UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE CIENCIAS BIOLÓGICAS

Departamento de Biología Vegetal I (Botánica y Fisiología Vegetal)

TESIS DOCTORAL

Estudio biosistemático y ecológico en eumycetozoa (amoebozoa): protostélidos y myxomycetes

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

María Aguilar González

Director

Carlos Lado Rodríguez

Madrid, 2012

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MEMORIA DE TESIS DOCTORAL
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MARÍA AGUILAR GONZÁLEZ

V. Bº Director: **Dr. CARLOS LADO RODRÍGUEZ**

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ESTUDIO BIOSISTEMÁTICO Y ECOLÓGICO EN EUMYCETOZOA (AMOEBOZOA): PROTOSTÉLIDOS Y MYXOMYCETES

Memoria para optar al grado de DOCTOR en CIENCIAS (Sección Biológicas) que presenta la Licenciada:

MARÍA AGUILAR GONZÁLEZ

Este trabajo ha sido dirigido por:
CARLOS LADO RODRÍGUEZ
(Real Jardín Botánico, CSIC).

MADRID
2012

Don Carlos Lado Rodríguez, Doctor en Farmacia e Investigador Científico del Real Jardín Botánico (CSIC) informa de que:

La memoria titulada **Estudio Biosistemático y Ecológico en Eumycetozoa (Amoebozoa): Protostélidos y Myxomycetes** que presenta María Aguilar González, Licenciada en Biología, para optar al grado de Doctor, ha sido realizada en el Real Jardín Botánico (CSIC) bajo su dirección, reuniendo todas las condiciones exigidas a los trabajos de tesis doctoral.

Madrid, 6 de Febrero de 2012

Fdo. Carlos Lado Rodríguez

AGRADECIMIENTOS

Ante todo, me gustaría agradecer a Carlos Lado el haberme dado la oportunidad de poder realizar la tesis doctoral bajo su dirección. Gracias por la confianza y el apoyo prestados durante estos años, y por el tiempo dedicado a la revisión de manuscritos y de esta memoria.

Quisiera también hacer extensivo este agradecimiento al resto de miembros del grupo, Diana Wrigley de Basanta, Eva García y Juan Carlos Hernández, por su trato cordial y afectuoso y por sus buenos consejos.

También quisiera expresar mi gratitud a los equipos de investigación que me han acogido en sus laboratorios durante las estancias breves, por la gran experiencia que ha supuesto poder trabajar con ellos y lo mucho que he aprendido.

En primer lugar el grupo de investigación de Fred Spiegel en la Universidad de Arkansas (EEUU): Lora Shadwick, John Shadwick, Matt Brown, George Ndiritu, Steve Stephenson, Carlos Rojas, Katie Winset y Jeff Silberman.

Y por otro lado el grupo de investigación de Tom Cavalier-Smith en la Universidad de Oxford (Reino Unido): Ema Chao, Anne-Marie Fiore-Donno, Cédric Berney, Libby Schnell, Rodri Lewis, Jojo Scoble, Ed Glücksman y David Bass.

Muchas gracias a todas las personas que han contribuido a la realización de este trabajo enviándome muestras de mixomicetes y sustratos para el cultivo de protostélidos: Marianne Meyer, Steve Stephenson, Lais de Holanda Cavalcanti, Roland McHugh y Cristina Gomes.

Gracias también a los técnicos de laboratorio del Real Jardín Botánico, Fátima Durán, Gemma Andreu, Gillermo Sanjuanbenito, que han facilitado mucho el trabajo al ayudar en la elaboración de medios de cultivo y en la extracción de ADN de las muestras de mixomicetes. También gracias a Yolanda Ruiz por su buen hacer en el uso del microscopio electrónico.

También me gustaría dar las gracias al resto del personal del Real Jardín Botánico por haber proporcionado un excelente ambiente de trabajo. Gracias en especial a Isabel Marques, Andrea Sánchez, Jano González, Cajsa-Lisa Anderson, Emilio Cano, Isabel Sanmartín, Javier Fuertes, Luis Valente, Angélica Bello, Carlos Rodríguez, Jose Luis Blanco, Juan Carlos Zamora, Elena Amat, Bárbara Ríos, Arantxa Martínez, Raquel Pino, Paco Calonge… y un largo etc.

Quisiera también expresar mi agradecimiento a todas las personas que me han aportado nuevas ideas, críticas constructivas, y tantos buenos consejos en los congresos a los que he asistido. En este apartado me gustaría dar especialmente las gracias a los miembros de ISOP y ISEP.

También muchas gracias los revisores anónimos por el tiempo y el interés que han invertido ayudando a mejorar mis artículos.

Gracias a quien corresponda por la beca FPI que me fue concedida y sus ayudas para estancias breves, a los proyectos de investigación que han financiado mi trabajo, y a las becas de viaje que me han permitido asistir a varios congresos.

Por último, y de manera muy especial, muchas gracias a las personas que han estado siempre ahí dándome su apoyo: mis padres y el resto de la familia, y también Mar, Lalu, Tess, Jorge, Alba, Angelo, Naira, Sara, Maken, Álvaro, Luisito, Bea y Miguel.

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Introducción General

Los Eumycetozoa son un grupo de microorganismos eucariotas heterótrofos, abundantes y prácticamente ubicuos en las comunidades de descomponedores terrestres (Spiegel, 1990; Stephenson el al, 2008). Se trata de un conjunto de organismos probablemente con orígenes diversos, que comparten un modo de vida y ciertas características morfológicas comunes. Realizan una labor muy importante en los ecosistemas, ya que se alimentan de las bacterias y los hongos que descomponen los tejidos vegetales, controlando y regulando sus poblaciones (Feest, 1987). Quizás debido a su pequeño tamaño, que hace que la mayoría no puedan ser observados en detalle sin usar una lupa o un microscopio, su estudio se ha visto limitado y son todavía organismos muy desconocidos. Faltan datos aún sobre muchos aspectos básicos de su biología (ciclos vitales, mecanismos de reproducción sexual, transmisión y recepción de señales para la agregación, etc.) y apenas existe información sobre los patrones de distribución de las distintas especies, así como de sus preferencias ecológicas.

Desde el descubrimiento de los eumicetozoos éstos han sido tratados como organismos "enigmáticos" o "misteriosos", debido a que se consideraba que poseían características tanto de plantas, como de hongos y de animales (Martin, 1960). Su peculiar morfología caracterizada por la formación de cuerpos fructíferos y estados tróficos ameboides, ha dificultado su comparación con otros grupos y ha provocado que su clasificación haya variado mucho a lo largo del tiempo. A pesar de que su afinidad con los protistas ya fue propuesta por Haeckel (1866), los eumicetozoos han sido tradicionalmente estudiados por micólogos, y Fries (1829) los incluyó dentro de los gasteromycetes. De Bary (1859) estudió por primera vez sus ciclos vitales y creó la nueva clase Mycetozoa para agrupar a mixomicetes y acrásidos, considerando que habían evolucionado independientemente tanto de plantas como de hongos verdaderos, y que se encontraban más cerca de los protistas. También incluyó a *Labyrinthula* y *Plasmodiophora* como posibles Mycetozoa. Más tarde, Olive (1975) estableció el taxón Eumycetozoa para incluir a los hongos mucilaginosos con células tróficas que producían "pseudópodos filosos" (de aquí en adelante subseudópodos filosos), excluyendo así a los acrásidos y otros organismos plasmodiales. El nombre Mycetozoa se sigue todavía usando, y se emplea en unos casos como sinónimo de Eumycetozoa, y en otros en el sentido que le dio de Bary. Para evitar confusiones, en esta memoria se ha decidido usar la terminología de Olive (1975), denominando eumicetozoos al grupo formado por mixomicetes, dictiostélidos y protostélidos.

Estos hongos mucilaginosos se distinguen de los hongos verdaderos en varios aspectos:

• Los eumicetozoos se alimentan de manera fagotrófica, mientras que los hongos nunca lo hacen así.
• Los eumicetozoos tienen crestas mitocondriales tubulares y los hongos las tienen planas.
• Los eumicetozoos no poseen paredes celulares en su fase trófica, mientras que los hongos típicamente, aunque no siempre, tienen paredes de quitina en su fase trófica.
• Filogenias moleculares basadas en el rADN muestran claramente que los hongos verdaderos forman un grupo monofilético muy alejado en su posición en el árbol de los eumicetozoos, que aparecen entre otros protistas.

No obstante, la nomenclatura que se emplea para los eumicetozoos se rige por el código internacional de nomenclatura para algas, hongos y plantas (ICN), previamente conocido como Código Internacional de Nomenclatura Botánica (ICBN), y la terminología usada para referirse a las estructuras morfológicas es micológica. También debido al enfoque tradicionalmente micológico de su estudio, la descripcion e identificación de las especies se basa principalmente en los caracteres morfológicos del cuerpo fructífero, y hasta hace poco no se ha prestado atención a otros estadíos de su ciclo vital, como amebas, ameboflagelados, quistes y plasmodios.

Todo este marco histórico dificulta el trabajo con estos organismos, ya que la bibliografía previa existente sobre el grupo utiliza metodologías, y nomenclatura propias del campo de la micología, y toda la bibliografía sobre sus grupos afines que permite estudiarlos en un marco más general ha sido realizada con perspectivas más microbiológicas.

En esta memoria se aborda el estudio de los factores ecológicos que condicionan la distribución de diferentes organismos pertenecientes a Eumycetozoa, prestando especial atención a las amebas protosteloides, el grupo más variado y de clasificación más complicada, y a los mixomicetes, el grupo más extensamente estudiado y sobre el que disponemos de más información. Asimismo, se aporta una contribución al estudio sistemático de estos organismos.

SISTEMÁTICA

La fructificación agregativa no es un carácter exclusivo de los eumicetozoos. Varios grupos de microorganismos terrestres con muy diversos orígenes han desarrollado independientemente la capacidad de agregarse para formar fructificaciones y dispersarse mediante esporas transportadas por el viento, el agua o los animales (Figura 1). A continuación se describen sucintamente algunos ejemplos.

Las myxobacterias son organismos procariotas que se encuentran típicamente en el suelo y que pertenecen al grupo de las proteobacterias. Las bacterias de este tipo se agregan, desplazándose y alimentándose en masa, y pueden formar cuerpos fructíferos con esporas (mixosporas) esféricas con gruesas paredes (Dawid, 2000).

En 1978 se describió una especie de ciliado llamado *Sorogena stoianovitchae* (Olive, 1978), un curioso organismo perteneciente a Chromalveolata (Lasek-Nesselquist & Katz, 2001), capaz de agregarse, conservando la individualidad de las células, para formar fructificaciones en las que el estípite está formado por secreciones.

Bajo la denominación informal de hongos mucilaginosos (slime moulds en terminología inglesa) se agrupan organismos de muy distinto origen, aunque todos ellos tienen la particularidad de que en sus ciclos vitales aparecen células ameboides o ameboflageladas, además de los ya nombrados cuerpos fructíferos. Entre ellos destacan los acrásidos y los eumicetozoos.

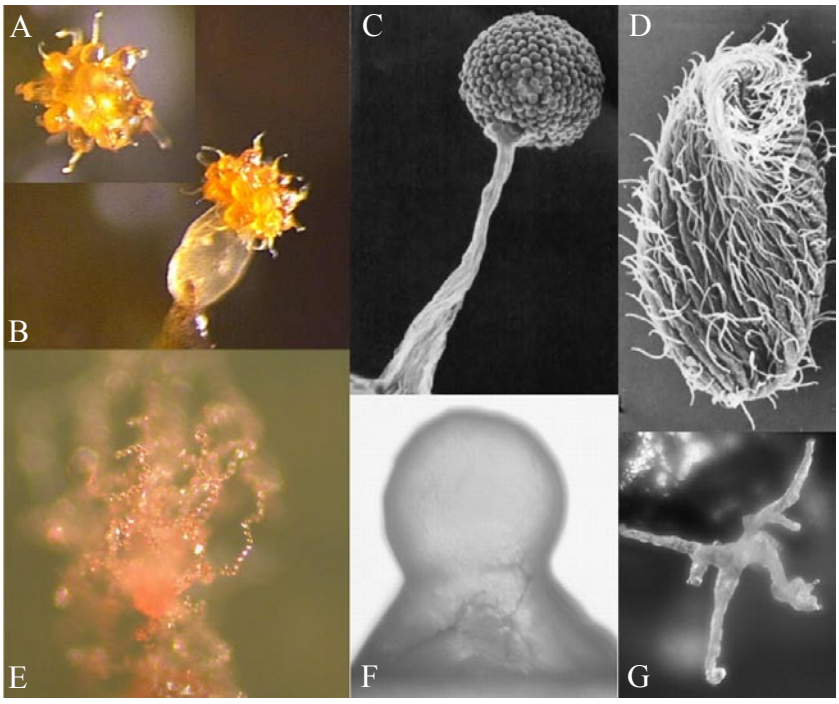


Figura 1 – Microorganismos terrestres que fructifican pero no son considerados eumicetozoos. A, B: Myxobacteria (microbelibrary.org), C: cuerpo fructífero de *Sorogena stoianovitchae* (Olive & Blanton, 1980), D: célula individual ciliada de *S. stoianovitchae* (Bardele et al, 1991), E: *Acrasis rosea* (wikipedia.org), F: *Fonticula alba* (Brown et al, 2009), G: *Copromyxa protea* (Brown et al, 2011a).

Olive (1975) define los acrásidos como un grupo de organismos ameboides con pseudópodos redondeados y que nunca forman flagelos. Cuando se agregan, las células conservan su individualidad y forman cuerpos fructíferos en los que todas las células siguen vivas. Son un grupo artificial cuyos organismos pertenecen a linajes de eucariotas muy distantes entre si: *Acrasis rosea*, y *Acrasis helenhemmesae* pertenecen al grupo de protistas Heterolobosea (Brown et al, 2010), *Copromyxa protea* es un amebozoo (Brown et al, 2011a), *Fonticula alba* es un opistoconto (Brown et al, 2009), y *Guttulinopsis* pertenece al supergrupo Rhizaria (Brown et al, 2011b).

Los eumicetozoos agrupan a mixomicetes, dictiostélidos y protostélidos, y son un grupo cuya monofilia está actualmente en duda. Son amebozoos que tienen en común el poseer subpseudópodos puntiagudos (pseudópodos puntiagudos según Olive, 1975) y mitocondrias con crestas tubulares (Dykstra, 1977), aunque estos caracteres no son sinapomorfías de los eumicetozoos (Stewart & Mattox, 1980; Page, 1988; Spiegel, 1990, 1991), sino que aparecen en otros grupos de eucariotas.

Eumycetozoa

Desde que se comenzaron a estudiar los hongos mucilaginosos, se tendió a pensar que todas las amebas de este grupo y todos los cuerpos fructíferos eran homólogos (Gray & Alexopoulos, 1968; Spiegel et al, 1995). Sin embargo, Olive (1975) adoptó una postura más escéptica y se mostró en contra de la idea de que todos los hongos mucilaginosos formaran un grupo monofilético, pero sin embargo defendió la existencia de un grupo Eumycetozoa monofilético, que incluiría exclusivamente a mixomicetes, dictiostélidos y protostélidos, dejando fuera a los acrásidos (Figura 2).

Con los datos procedentes de filogenias moleculares, también se ha puesto en duda que los distintos grupos de eumicetozoos estén directamente emparentados entre si. Sin embargo, aunque las relaciones existentes entre los grupos no estén todavía claramente establecidas, los últimos datos parecen indicar que tanto los mixomicetes, como los dictiostélidos y los protostélidos pertenecen a Amoebozoa (Baldauf et al, 2000; Fiore-Donno et al, 2010; Shadwick et al, 2009a).

Amoebozoa (Cavalier-Smith, 1998) es un supergrupo de eucariotas (Figura 3) que incluye a gran parte de los organismos con movimiento ameboide, basado en la actividad de actina-miosina del citoesqueleto. Agrupa a organismos como las amebas lobosas tanto desnudas (Smirnov et al, 2005) como tecadas (Nikolaev et al, 2005), arqueamebas y eumicetozoos (Cavalier-Smith, 1998), junto con el uniciliado *Phalansterium solitarium* (Cavalier-Smith et al, 2004), el multiciliado *Multicilia marina* (Nikolaev et al, 2006) y *Breviata anathema* (Minge et al, 2009). La mayoría son unicelulares y muchos son de vida libre siendo comunes en el suelo y en los hábitats acuáticos, algunos se encuentran en simbiosis con otros organismos, mientras que otros son parásitos. El movimiento ameboide lo realiza toda la célula, por lo que las amebas no suelen poseer una forma estable ni orgánulos locomotores diferenciados, haciendo que su identificación y clasificación desde el punto de vista morfológico sea muy complicada. En el pasado se intentó clasificarlos basándose en la morfología de las formas locomotoras (Schaeffer, 1926), en los diferentes patrones de flujo citoplásmi-

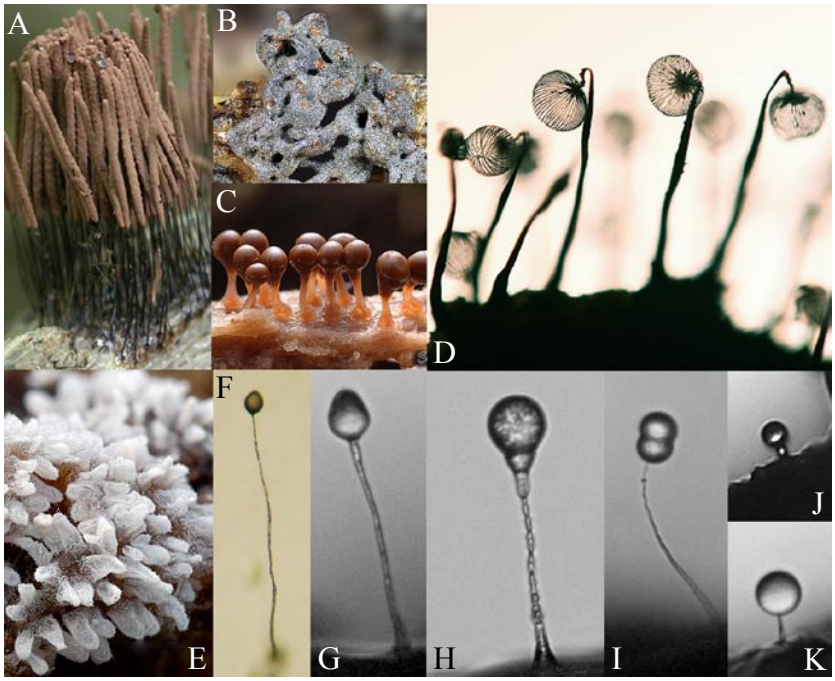


Figura 2 – Cuerpos fructíferos de eumicetozoos. A-D: mixomicetes (J. Arrabal) E: *Ceratiomyxa*, F: dictiostélido, G-K: protostélidos (M.Aguilar).

co (Jahn & Bovee, 1965; Jahn et al, 1974) y por sistemas basados en combinar caracteres visualizados por microscopía óptica y electrónica junto con aspectos de la biología y la fisiología de las especies (Page, 1987, 1988, 1991). Estos métodos han facilitado el trabajo, pero sigue siendo muy dificil tanto la identificación de las especies, como el comprender las relaciones entre grupos, por lo se se hace imprescindible el uso de caracteres moleculares.

La mayoría de los eumicetozoos (mixomicetes, dictiostélidos y protostélidos) suelen aparecer junto con las arqueamebas en Conosa (Cavalier-Smith, 1998; Smirnov et al, 2005). Tanto los dictiostélidos como los mixomicetes son grupos monofiléticos (Baldauf et al, 2000; Fiore-Donno et al, 2010a), pero las amebas protosteloides podrían ser polifiléticas (Shadwick et al, 2009a; Fiore-Donno et al, 2010a; Lahr et al, 2011a), perteneciendo a muy diversos grupos de organismos dentro de Amoebozoa. Sin embargo sus posiciones relativas dentro de los amebozoos no están del todo claras y varían en distintos estudios. La monofilia del grupo formado por los mixomicetes y los dictiostélidos ha sido demostrada por análisis basados en EF-1α (Baldauf & Doolittle, 1997) y por análisis filogenómicos (Bapteste et al, 2002; Mingue et al, 2009), pero está en entredicho puesto que no aparece en todos los árboles (Cavalier-Smith et al, 2004; Shadwick et al, 2009a; Fiore-Donno et al, 2010a). Debido al desconocimiento que todavía existe sobre estos organismos, algunos autores han afirmado que el establecimiento de una clasificación formal no es apropiado por el momento (ver por ejemplo Adl et al, 2005) y que es necesario que se esclarezcan las relaciones filogenéticas entre los grupos antes de construir un nuevo sistema de clasificación.

Por lo que parece, los eumicetozoos son organismos que surgieron hace mu-

chos millones de años. Mediante el uso de un reloj molecular relajado se ha datado la divergencia de dictiostélidos y mixomicetes entre hace 800 y hace 1300 millones de años (Parfrey et al, 2011). Sin embargo, los fósiles de mixomicetes que se conocen son mucho más recientes: cuerpos fructíferos de una especie de *Stemonitis* (Dömke, 1952), cuerpos fructíferos de *Arcyria* (Dörfelt et al, 2003) en ámbar del Báltico del Eoceno, un plasmodio conservado en ámbar del Eoceno-Oligoceno en la República Dominicana (Waggoner & Poinar, 1992), y esporas fósiles del Oligoceno y el Pleistoceno (Graham, 1971).

Los eumicetozoos presentan la ventaja respecto a otros amebozoos de que, al ser la morfología del cuerpo fructífero más estable que la de las células ameboides, son mucho más sencillos de identificar. Esto, junto con sus muy diversas formas de vida y con sus distintos orígenes evolutivos distribuidos a lo largo de todo el supergrupo, hace que puedan ser buenos candidatos para ser usados como organismos modelo para el estudio de los patrones generales en Amoebozoa.

Dictiostélidos

Los dictiostélidos, también llamados hongos mucilaginosos celulares o amebas sociales, aparecen con frecuencia en el humus de bosques. Requieren una temperatura moderada, altos niveles de oxígeno en el suelo, niveles medios de humedad, y bacterias como alimento, prefiriendo medios ligeramente ácidos (Romeralo, 2007).

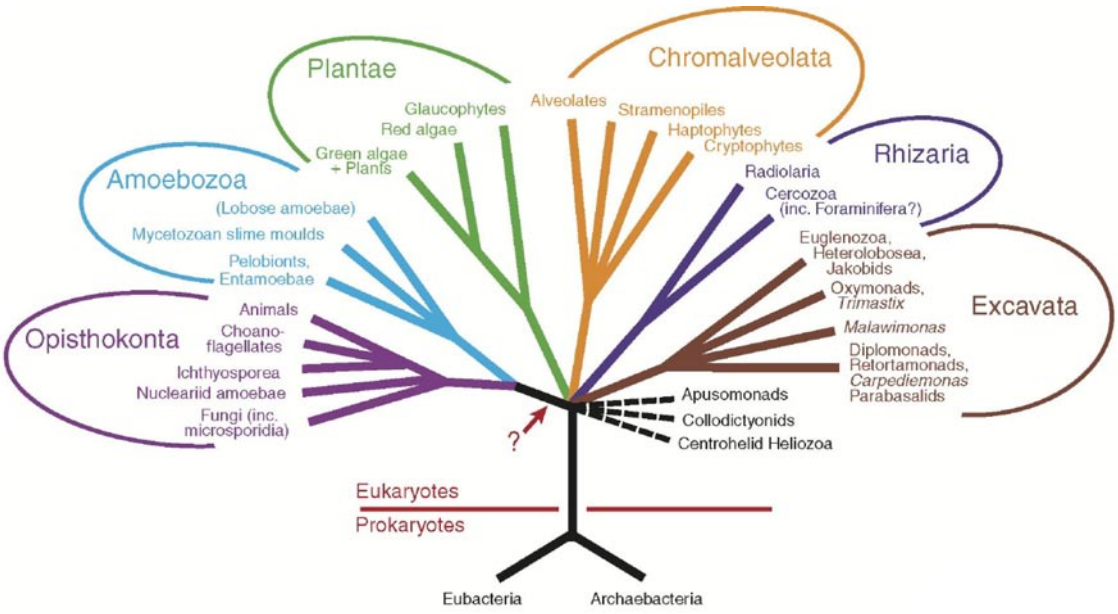


Figura 3 – Árbol que representa la organización de de los organismos eucariotas en seis grandes grupos. Las relaciones entre la mayoría de los grupos principales y la posición de la raíz del árbol se muestran como no resueltos. La flecha indica una posible ubicación de la raíz, basada en datos sobre fusión de genes (Simpson & Roger, 2004).

Debido a su tamaño microscópico, es necesario realizar cultivos con el material recolectado en el campo para poder detectarlos. Hasta ahora se han descrito alrededor de 100 especies pertenecientes a este grupo de organismos (Cavender, 1990; Lado, 2005-2011), que parecen distribuirse por todo el mundo, aunque el número de especies encontradas suele disminuir cuando aumenta la latitud o la altitud (Cavender, 1973).

Su ciclo de vida alterna varias fases. La primera fase está constituída por células ameboides haploides independientes que se van dividiendo. Cuando el alimento se agota, forman quistes (microquistes), se reproducen sexualmente (formando macroquistes) o bien fructifican. La fructificación está precedida por la agregación de las amebas para formar estructuras en las que las células no pierden su individualidad, llamadas pseudoplasmodios, que se desplazan y alimentan en bloque. En un determinado momento el pseudoplasmodio se desarrolla para dar lugar a un cuerpo fructífero, que está formado por distintos tipos de células especializadas con distintas funciones. Durante este proceso, las células encargadas de formar el estípite mueren. La reproducción sexual se inicia con la fusión de las amebas dos a dos, dando lugar a células diploides que se dividen por meiosis y luego se agregan para formar un quiste (macroquiste). Cuando las condiciones ambientales mejoran, los quistes germinan liberando amebas haploides.

El estudio de los dictiostélidos no es abordado en el presente trabajo, por lo que no nos extenderemos en más detalles. Para más información sobre ellos se recomienda consultar Romeralo (2007), y los trabajos de Olive (1975), Raper (1984) y Cavender (1990).

Myxomycetes

Los mixomicetes son el grupo de eumicetozoos con mayor número de especies, con cerca de 1000 taxones descritos (Lado, 2005-2011). También son llamados myxogástridos u hongos mucilaginosos plasmodiales. Son organismos que viven en muy diferentes tipos de hábitats terrestres, desde desiertos hasta ambientes de alta montaña, pasando por bosques tropicales y templados de todo tipo, zonas de matorral, pastizales, etc. Sus fructificaciones pueden aparecer sobre sutratos tan diversos como ramas caídas, hojas secas, corteza de plantas vivas, humus y excrementos de animales. Los cuerpos fructíferos (también llamados esporóforos) de mayores dimensiones pueden detectarse a simple vista, por lo que es posible recolectarlos directamente en el campo. También responden satisfactoriamente al cultivo de sustratos en cámara húmeda, lo que permite identificar las especies con cuerpos fructíferos de menor tamaño (menos de 500 μm) o las que se encuentran en otros estadíos de su ciclo vital, generalmente microscópicos (mixamebas, células ameboflageladas, microquistes, esclerocios), en el momento de recoger el material.

Su ciclo vital comienza cuando las esporas germinan y liberan protoplastos que, dependiendo del nivel de humedad, darán lugar a amebas (myxamebas) o ameboflagelados haploides, generalmente con 2 flagelos, que se alimentan de bacterias, levaduras y esporas de hongos, y se dividen por mitosis abiertas. La ultraestructura de los ameboflagelados es prácticamente idéntica en todas las especies estudiadas (Ishigami, 1977; Wright et al, 1979). Bajo condiciones desfavorables las células forman quistes (microquistes), que pueden desenquistarse posteriormente

originando de nuevo células ameboides o ameboflageladas. Las células ameboides pueden en un determinado momento fusionarse, dando lugar a zigotos diploides.

En determinadas circunstancias los zigotos diploides se agregan, perdiendo su individualidad como células pero sin que sus núcleos lleguen a fusionarse, dando lugar a plasmodios multinucleados. Los plasmodios crecen y se pueden fusionar entre sí, formando plasmodios de mayor tamaño. Mientras tanto los núcleos se dividen por mitosis cerrada de forma sincrónica, y el protoplasma puede fluir en forma de corrientes rítmicas bidireccionales. En condiciones desfavorables, el plasmodio puede formar quistes (esclerocios) o fructificaciones.

Las fructificaciones pueden adquirir formas y tamaños muy diversos variando según la especie: pueden conservar la forma del plasmodio (plasmodiocarpos), concentrar todo el plasmodio en una o unas pocas masas redondeadas (etalios), o fragmentarse en numerosos cuerpos fructíferos sésiles o estipitados (esporocarpos) (Lado & Pando, 1997). Las esporas casi siempre están marcadamente ornamentadas (Frederick, 1990), con la excepción del género *Echinostelium*. Durante la formación del cuerpo fructífero, los núcleos que darán lugar a las esporas se dividen por meiosis y luego se recubren por una pared resistente. Las fructificaciones ya maduras están formadas por las esporas haploides, y un conjunto de secrecciones celulares que forman diferentes estructuras de soporte: un estípite que eleva las esporas por encima del sustrato, una vaina externa (peridio), y una red de fibras estériles que rodean a las esporas (capilicio).

Con el uso de caracteres morfológicos de sus cuerpos fructíferos, los mixomicetes se han clasificado en 5 órdenes: Echinosteliales, Trichiales, Liceales, Stemonitales y Physarales (Lado & Pando, 1997). Los Ceratiomyxales han sido considerados como un sexto orden de mixomicetes por muchos autores, pero en esta memoria serán tratados con más detalle en la sección dedicada a los protostélidos (ver más adelante) teniendo en cuenta las clasificaciones realizadas por Olive (1975) y Spiegel (1990). Los principales caracteres que distinguen a los cinco órdenes considerados aquí son el color y la morfología de las esporas, la existencia o no de capilicio, su color, forma y ornamentación, y la presencia o no de depósitos calcáreos en algunas de sus extructuras, como peridio, capilicio, estípite y columela. También existen diferencias en las formas de desarrollo de las fructificaciones y la morfología de los plasmodios (Alexopoulos, 1969). Los caracteres distintivos de los órdenes son:

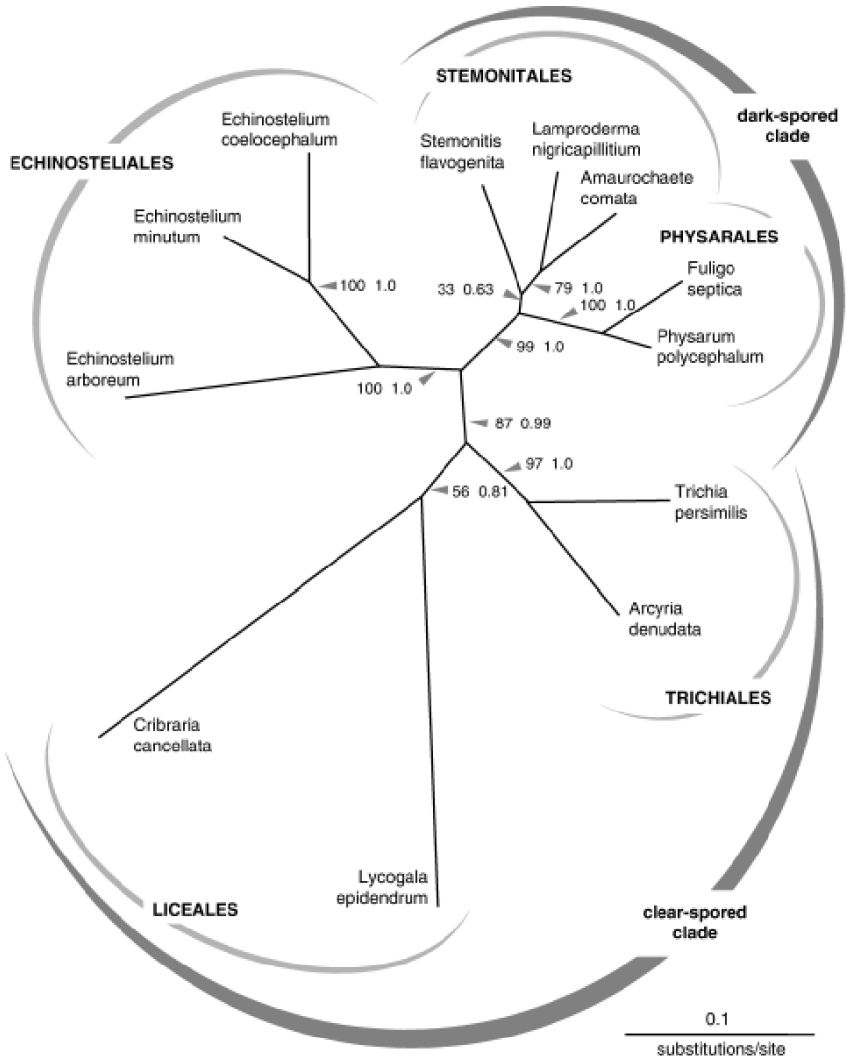
• Echinosteliales: forman esporocarpos estipitados de pequeño tamaño (<1,3 mm de altura, generalmente <0,5 mm de altura), por lo que es necesario el uso de una lupa para observarlos. Carecen de depósitos calcáreos, las esporas están poco o nada ornamentadas y son de colores claros en la mayoría de las especies (de hialinas a amarillentas, rosadas o pardo grisáceas). Pueden tener o no capilicio. Sus plasmodios son pequeños, hialinos y con pocos núcleos (protoplasmodios). Incluyen dos familias: Echinosteliaceae, siempre con capilicio incoloro o muy pálido y Clastodermataceae, con capilicio de color pardo oscuro.
• Trichiales: forman cuerpos fructíferos estipitados o sésiles, generalmente sin depósitos calcáreos, de más de 0,5 mm de altura y con capilicio abundante formado por fibras huecas o macizas. Las

esporas son de colores claros (de amarillas a rosadas o rojizas). Pueden presentar faneroplasmodios o afanoplasmodios.

• Liceales: poseen cuerpos fructiferos estipitados o sésiles, de tamaño muy variable de 0,1-500 mm, con o sin depósitos calcáreos, con esporas de colores claros, y sin capilicio. Poseen protoplasmodios.

• Stemonitales: sus fructificaciones presentan esporas de colores oscuros (pardas, violáceas o negruzcas) y capilicio bien desarrollado, pardo, reticulado o dicotómico. Sin depósitos calcáreos. Plasmodios de tipo afanoplasmodio.

Figura 4 – Filogenia de los mixomicetes. Basada en secuencias del factor de elongación 1-alfa (EF-1α) y el ADN que codifica para la subunidad pequeña del ribosoma (SSU rADN). Incluye representantes de los cinco órdenes de mixomicetes, que se agrupan formando el clado de esporas oscuras y el clado de esporas claras (Fiore-Donno et al, 2005).



• Physarales: fructificaciones con esporas de colores oscuros (pardas, violáceas o negruzcas), y capilicio bien desarrollado, pardo o blanquecino, a menudo reticulado. Con depósitos calcáreos en alguna parte de los cuerpos fructíferos. Plasmodio de tipo faneroplasmodio.

Mediante análisis filogenéticos, aunque todavía con pocos taxones analizados y casi siempre usando uno o dos marcadores, se ha visto que las clases parecen mantenerse, pero además se ha podido observar que existen tres grandes subdivisiones en los mixomicetes (Fiore-Donno et al, 2005): Echinosteliales como grupo basal, con esporas claras y oscuras y fructificaciones más sencillas, un clado formado por Trichiales y Liceales, con esporas claras, y un tercer clado hermano del anterior que agrupa a las clases Stemonitales y Physarales, con esporas oscuras (Figura 4). Posteriormente y ampliando el número de especies analizadas, se ha visto que el orden Stemonitales podría ser parafilético (Fiore-Donno et al, 2008). En otros trabajos basados en análisis filogenéticos se ha observado también que entre los mixomicetes se intercalan varios tipos de amebas que no fructifican, y que hasta ahora se habían clasificado dentro del género *Hyperamoeba* (Fiore-Donno et al, 2010b). También se incluye en los mixomicetes el misterioso organismo *Semimorula liquescens* (Fiore-Donno et al, 2009) del que hasta hace poco se desconocían sus afinidades.

Debido a que todavía se necesitan muchos más datos para clarificar la filogenia de los mixomicetes tanto a nivel profundo, como a nivel de géneros y familias, quizás la mejor manera de clasificar a estos organismos por el momento sea usar el sistema sin categorías propuesto por Adl et al (2005).

Protostélidos

Los protostélidos o amebas protosteloides producen cuerpos fructíferos sencillos, compuestos por un estípite acelular y una o unas pocas esporas. Es el grupo que tiene la mayor diversidad de ciclos vitales y morfología de los estados tróficos. Parecen poseer una amplia distribución, ya que muchas de las especies han sido encontradas en partes del mundo muy distantes entre si (Olive, 1975; Moore & Spiegel, 1995, 2000a, b, c; Moore et al, 2000; Spiegel & Stephenson, 2000; Shadwick & Stephenson, 2004; Tesmer et al, 2005; Spiegel et al, 2007), tanto en climas templados como en tropicales, desérticos o fríos. Han sido aislados de gran variedad de sustratos como restos vegetales, humus, excrementos o corteza de plantas vivas (Olive, 1975). Son todos heterótrofos y actúan como depredadores en su ambiente, alimentándose de organismos descomponedores como bacterias, levaduras y esporas de hongos filamentosos. Los protostélidos son los eumicetozoos de menor tamaño (< 300 μm de altura), lo que dificulta su observación y estudio haciendo necesario el uso de cultivos y del microscopio para detectarlos. Estos organismos fueron descubiertos hace tan solo cinco décadas (Olive & Stoianovitch, 1960), y hasta ahora sólo se conocen 33 especies (Spiegel et al, 2007).

Las amebas protosteloides resentan una gran diversidad en sus ciclos vitales, y los estados tróficos varían desde formas ameboides uninucleadas a células ameboflageladas o plasmodios multinucleados y reticulados, y no todas las especies tienen la capacidad de formar flagelos o plasmodios. Los plasmodios de los protostélidos se diferencian de los de mixomicetes en que los primeros no poseen flujo rítmico y reversible del protoplasma, aunque sí muestran

cierto grado de sincronización en la división nuclear (Olive, 1975). La morfología tanto del plasmodio como de las amebas es muy variable. No está claro si todos se reproducen sexualmente ni por qué mecanismos lo hacen, aunque se han detectado indicios de meiosis (Spiegel, 1990). Además, sus ciclos vitales complejos con varios tipos de células tróficas parecen congruentes con la presencia de reproducción sexual (Lahr et al, 2011b).

El primer protostélido se descubrió accidentalmente en 1960, cuando sus autores estaban tratando de aislar acrásidos. Este nuevo organismo recibió el nombre de *Protostelium mycophaga* L.S.Olive & Stoian. y fue inicialmente clasificado como un dictiostélido (Olive & Stoianovitch, 1960). Con el descubrimiento de nuevos protostélidos, se vio la necesidad de crear primero una familia, y más tarde una nueva subclase para ellos (Olive, 1975). Durante casi 25 años Olive y Stoianovitch trabajaron en el grupo, desvelando la diversidad morfológica de los protostélidos y su ubicuidad dentro de la comunidad de descomponedores. La mayoría de los trabajos sobre protostélidos posteriores han sido realizados por discípulos de Olive, pero el interés en el grupo está empezando a crecer entre otros científicos no directamente relacionados con él (Spiegel, 1990).

Spiegel realizó numerosos estudios sobre la ultraestructura de los aparatos flagelares de estos organismos (Spiegel, 1981a b, 1982; Spiegel et al, 1986; Spiegel & Feldman, 1988). Gracias a sus aportaciones conocemos que los protostélidos flagelados presentan distintos aparatos flagelares, variando tanto el número de flagelos, como la disposición de las raíces del aparato flagelar y su vinculación con el núcleo.

Otro dato interesante es la presencia de celulosa en algunas estructuras. Los estípites y las paredes de esporas y quistes a menudo se vuelven azuladas en cloruro de zinc y los estípites de *Ceratiomyxella tahitiensis* se tornan de color azul cuando se testan con yoduro de potasio y ácido sulfúrico concentrado (Olive, 1975).

A continuación se ofrece un listado con las 33 especies de protostélidos descritas hasta la fecha, incluyendo las correcciones nomenclaturales propuestas por Lado (2005-2011):

Cavostelium apophysatum L.S.Olive, Mycologia 56(6):886 (1965 (“1964”))

Ceratiomyxella tahitiensis L.S.Olive & Stoian., Amer. J. Bot. 58(1):32 (1971a)

Clastostelium recurvatum L.S.Olive & Stoian., Trans. Brit. Mycol. Soc. 69(1):83 (1977)

Echinosteliopsis oligospora Reinhardt & Olive, Mycologia 58(6):967 (1967 (“1966”))

Echinostelium bisporum (L.S.Olive & Stoian.) K.D.Whitney & L.S.Olive, in Whitney, Bennett & Olive, Mycologia 74(4):680 (1982)
≡ *Cavostelium bisporum* L.S. Olive & Stoian., Mycologia 58(3):440 (1966)

Endostelium amerosporum L.S.Olive, in Olive, Bennett & Deasey, Mycologia 76(5):886 (1984)

Endostelium zonatum (L.S.Olive & Stoian.) W.E.Benn.& L.S.Olive, in Olive, Bennett & Deasey, Mycologia 76(5):891 (1984)
≡ *Protostelium zonatum* L.S. Olive & Stoian., Amer. J. Bot 56(9):985 (1969)

Microglomus paxillus L.S.Olive & Stoian., J. Protozool. 24(4):485 (1977)

Nematostelium gracile (L.S.Olive & Stoian.) L.S.Olive & Stoian., in Olive, Bot. Rev. 36(1):68 (1970) [como *gracilis*]
≡ *Schizoplasmodium gracile* L.S. Olive & Stoian., J. Protozool. 13:168 (1966)

Nematostelium ovatum (L.S.Olive & Stoian.) L.S.Olive & Stoian., in Olive, Bot. Rev. 36(1):68 (1970)
≡ *Schizoplasmodium ovatum* L.S. Olive & Stoian., J. Protozool. 13:164 (1966)

Planoprotostelium aurantium L.S.Olive & Stoian., J. Elisha Mitchell Sci. Soc. 87(3):115 (1971b)

Protosporangium articulatum L.S.Olive & Stoian., J. Protozool. 19(4):570 (1972)

Protosporangium bisporum L.S.Olive & Stoian., J. Protozool. 19(4):565 (1972)

Protosporangium conicum W.E.Benn., Mycologia 78(5):857 (1986)

Protosporangium fragile L.S.Olive & Stoian., J. Protozool. 19(4):568 (1972)

Protosteliopsis fimicola (L.S.Olive) L.S.Olive & Stoian., Mycologia 58:454 (1966)
≡ *Protostelium fimicola* L.S. Olive, Amer. J. Bot. 49(3):301 (1962)

Protostelium arachisporum L.S.Olive, Amer. J. Bot 49(3):301 (1962)

Protostelium mycophagum L.S.Olive & Stoian., Bull. Torrey Bot. Club 87(1):12 (1960) [como *mycophaga*]

Protostelium nocturnum Spiegel, Mycologia 76(3):443 (1984)

Protostelium okumukumu Spiegel, Shadwick & Hemmes, Mycologia 98(1):151 (2006)

Protostelium pyriforme L.S. Olive & Stoian., Amer. J. Bot. 56(9):987 (1969) [como *pyriformis*]

Schizoplasmodiopsis amoeboidea L.S.Olive & K.D.Whitney, Mycologia 74(4):655 (1982)

Schizoplasmodiopsis micropunctata L.S.Olive & Stoian., Mycologia 67(6):1097 (1975)

Schizoplasmodiopsis pseudoendospora L.S.Olive, M.Martin. & Stoian., in Olive, Mycologia 59(1):19 (1967)

Schizoplasmodiopsis reticulata L.S.Olive & Stoian., Mycologia 67(6):1089 (1975)

Schizoplasmodiopsis variabilis L.S. Olive, Trans. Brit. Mycol. Soc. 84(3):539 (1985)

Schizoplasmodiopsis vulgaris L.S.Olive & Stoian., Mycologia 67(6):1092 (1975) [como *vulgare*]

*Schizoplasmodium cavostelioide*s L.S.Olive & Stoian., Amer. J. Bot 53(4):344 (1966)

Schizoplasmodium obovatum L.S.Olive & Stoian., Amer. J. Bot 63(10):1387 (1976)

Schizoplasmodium sechellarum L.S.Olive & Stoian., Amer. J. Bot. 63(10):1387 (1976)

Soliformovum expulsum (L.S.Olive & Stoian.) Spiegel, in Spiegel, Gecks & Feldman, J. Eukaryotic Microbiol. 41(5):518 (1994)
≡ *Protostelium expulsum* L.S. Olive & Stoian., Trans. Brit. Mycol. Soc. 76(2):303 (1981)

Soliformovum irregulare (L.S.Olive & Stoian.) Spiegel, in Spiegel, Gecks & Feldman, J. Eukaryotic Microbiol. 41(5):518 (1994) [como *irregularis*]
≡ *Protostelium irregulare* L.S. Olive & Stoian., Amer. J. Bot. 56(9):983 (1969)

Tychosporium acutostipes Spiegel, D.L. Moore & J.Feldman, Mycologia 87(2):265 (1995)

Los protostélidos han sido clasificados de varias formas diferentes desde que se conocen. La primera clasificación (Tabla 1) fue realizada por Olive (1975, 1982), aunque afirmando él mismo que se trataba de un sistema artificial y con fines prácticos, pues seguramente no obedecía a las verdaderas relaciones evolutivas existentes entre los organismos. Esta clasificación se estableció para facilitar el estudio del grupo, con el fin de poder usarla hasta que se realizara un estudio más profundo que clarificara las relaciones entre los organismos.

El género *Ceratiomyxa* incluye organismos formados por esporocarpos microscópicos con una sola espora que se sitúan sobre columnas plasmodiales que se solidifican al secarse. Estos organismos se consideraban tradicionalmente como un grupo de mixomicetes, pero fueron incluidos dentro de los protostélidos en la clasificación de Olive (1975, 1982) debido a la similitud de las estructuras que soportan las esporas con los esporocarpos de protostélidos. Spiegel (1990) también los consideró en su clasificación de los protostélidos, incluyéndolos en su grupo Va junto con *Clastostelium* y *Protosporangium*. Sin embargo, los estudios filogenéticos realizados recientemente ponen en duda su parentesco directo con el resto de protostélidos, y en algunos casos aparecen como grupo hermano de los mixomicetes (Fiore-Donno et al, 2010a). Podemos afirmar que hoy en día sus afinidades evolutivas no están todavía clarificadas. No obstante, la tendencia actual es considerarlos como un grupo aparte con respecto al resto de las amebas protosteloides y los mixomicetes a la espera de clarificar su posición en el árbol de los amebozoos.

Spiegel (1990), basándose en los ciclos vitales y en sus estudios de las ultraestructuras de los ameboflagelados y la morfología de las amebas, propuso una nueva clasificación (Tabla 2) de las especies en grupos, esta vez con intención de detectar qué especies eran más cercanas evolutivamente entre sí. A esta clasificación fueron añadidos posteriormente nuevos datos sobre la morfología del nucleolo (Lindley et al, 2006).

Debido a la simplicidad de sus fructificaciones, las primeras hipótesis evolutivas sobre las amebas protosteloides afirmaban que organismos con este tipo de morfología habrían dado lugar tanto a mixomicetes como a dictiostélidos (Olive, 1975), suponiendo por tanto que al menos se trataba de un grupo parafilético. Durante los años 70 se realizaron numerosos estudios ul-

traestructurales que pusieron de manifiesto la diversidad morfológica de los estados tróficos de protostélidos (Hung & Olive, 1972a, b, 1973a, b). Con los nuevos datos aportados durante los años 80 y a la luz de todo el trabajo anterior, comenzó a sospecharse que el grupo podría ser polifilético (Olive 1982; Whitney & Bennett, 1984; Spiegel et al, 1995). Recientemente se ha publicado un análisis filogenético basado en SSU rDNA, y que incluye la gran mayoría de especies de protostélidos (Shadwick et al, 2009a). En este trabajo se muestra que los diferentes grupos de protostélidos no están directamente relacionados entre si, sino que aparecen entremezclados

Tabla 1 - Clasificación de los protostélidos según Olive (1975, 1982).

Familia	Género	Especies
Cavosteliidae (con ameboflagelados en su ciclo vital, esporocarpos individualizados)	***Cavostelium*** L.S. Olive, Mycologia 56(6):885 (1965 ("1964"))	*C. apophysatum*
	Protosporangium L.S. Olive & Stoian., J. Protozool. 19(4):563 (1972)	*P. bisporum* *P. fragile* *P. articulatum*
	Ceratiomyxella L.S. Olive & Stoian., Amer. J. Bot. 58(1):32 (1971a)	*C. tahitiensis*
	Planoprotostelium L.S. Olive & Stoian., J. Elisha Mitchell Sci. Soc. 87(3):115 (1971b)	*P. aurantium*
	Clastostelium L.S. Olive & Stoian., Trans. Brit. Mycol. Soc. 69(1):83 (1977)	*C. recurvatum*
	Echinostelium de Bary, in Rostafinski, Vers. Syst. Mycetozoen 7 (1873)	*E. bisporum*
Ceratiomyxidae (con ameboflagelados en su ciclo vital, esporocarpos agrupados sobre una estructura común)	***Ceratiomyxa*** J. Schröt., in Engler & Prantl, Nat. Pflazenfam. 1(1) :16 (1889)	*C. fruticulosa* *C. morchella* *C. sphaerosperma* *C. hemisphaerica*
Protosteliidae (sin amoeboflagelados en su ciclo vital, esporocarpos individualizados con una sola espora)	***Protostelium*** L.S. Olive & Stoian., Bull. Torrey Bot. Club 87(1):12 (1960)	*P. mycophaga* *P. nocturnum* *P. pyriformis* *P. irregularis* *P. expulsum* *P. arachisporum* *P. zonatum*
	Nematostelium L.S. Olive & Stoian., in Olive, Bot. Rev. 36(1):68 (1970)	*N. ovatum* *N. gracile*
	Schizoplasmodium L.S. Olive & Stoian., Amer. J. Bot 53(4):344 (1966)	*S. cavostelioides* *S. sechellarum* *S. obovatum*
	Protosteliopsis L.S. Olive & Stoian., Mycologia 58(3):452 (1966)	*P. fimicola*
	Schizoplasmodiopsis L.S. Olive, Mycologia 59(1):19 (1967)	*S. pseudoendospora* *S. vulgare* *S. reticulata* *S. micropunctata* *S. amoeboidea*
	Microglomus L.S. Olive & Stoian., J. Protozool. 24(4):485 (1977)	*M. paxillus*
Echinosteliopsidae (sin amoeboflagelados en su ciclo vital, esporocarpos individualizados con más de una espora)	***Echinosteliopsis*** Reinhardt & L.S. Olive, Mycologia 58(6):967 (1967 ("1966"))	*E. oligospora*

Tabla 2 - Clasificación de los protostélidos según Spiegel (1990) y modificada en Lindley et al (2006).

Grupo	Miembros	Ameboflagelado	Caracteres de los flagelados	Nucleolo	Caracteres importantes del grupo
I	***Planoprotostelium***	Sí	- Varias quinétidas por célula - Sin unión al núcleo	Único, central, esférico	- Morfología de la ameba y pigmentación naranja - Células preesporales elongadas - Presencia de MTOC
	***Protostelium* pp.**	No	NA		
II	***Ceratiomyxella***	Sí	- Normalmente una quinétida por célula - Con unión al núcleo - Con escamas	Único, central, esférico	- Morfología del plasmodio y mitosis - Patrón de formación de las células preesporales - Apófisis e Hilum
	Nematostelium Schizoplasmodium	No	NA		
III	***Soliformovum***	No	NA	Difuso	- Amebas flabeliformes - Múltiples nucleolos - Células preesporales en forma de "huevo frito"
IV	***Cavostelium***	Sí	- Varias quinétidas por célula - Sin unión al núcleo - Cobertura celular fibrosa	Único, central, esférico	- Amebas muy ramificadas - Plasmodios - Espinas poco transparentes a los electrones en la pared esporal
	***Schizoplasmodiopsis*, pp.**	No	NA		
Va	***Protosporangium Clastostelium Ceratiomyxa***	Sí	- Una o más quinétidas por célula - Al menos una quinétida unida al núcleo - Cobertura celular fibrosa	Único, central, esférico	- Estado flagelado presente por corto espacio de tiempo en el ciclo: después de la germinación de la espora - División del núcleo (¿meiosis?) en la espora o en las células preesporales, sobreviviendo todos los núcleos - Paredes de la espora lisas
Vb	***Echinostelium bisporum***	Sí	- Una quinétida por célula - Con unión al núcleo - Idéntico al de mixomicetes	Único, central, esférico	- Estado flagelado puede presentarse durante toda la fase trófica del ciclo - Meiosis en las esporas, sobreviviendo sólo un núcleo -Paredes de la espora ornamentadas como las de mixomicetes
VI	***Protosteliopsis fimicola***	No	NA	Único, central, esférico	- Mitocondrias con crestas tubulares
	Microglomus paxillus				
	Echinosteliopsis oligospora			Múltiples, periféricos	
	Schizoplasmodiopsis amoeboidea			Difuso	
VII	***Endostelium zonatum***	No	NA	Único, central, esférico	- Mitocondrias con crestas vesiculares - Amebas circulares y gruesas
	Protostelium arachisporum				

con diferentes grupos de amebozoos no fructificantes, y prácticamente repartidos por todo el supergrupo Amoebozoa (Figura 5). Estos resultados ponen en duda la validez del concepto de eumicetozoos monofiléticos, y también muestran a los mixomicetes y dictiostélidos como grupos monofiléticos no directamente relacionados entre si. Como resultado del trabajo de Shadwick et al (2009a) se obtuvieron los siguientes clados que prácticamente coinciden con los grupos de Spiegel (1990):

Clado Protosteloideo (Protosteliid Clade) - Grupo I

Este grupo incluye a *Protostelium mycophaga*, *Protostelium nocturnum*, *Protostelium okumukumu* y *Planoprotostelium aurantium* y aparece como grupo hermano del clado Protosporangioide. Estos organismos se caracterizan por poseer amebas con pigmentación naranja y forman subpseudópodos terminados en punta. Las especies que liberan sus esporas activamente, *Protostelium nocturnum* y *Protostelium okumukumu* aparecen en la base. *Planoprotostelium aurantium*, que es el único que produce células ameboflageladas, no es un linaje basal del que surgirían el resto de los organismos que habrían perdido la capacidad de formar flagelos como se había supuesto hasta ahora, sino que aparece entre las especies del género *Protostelium*.

Clado Schizoplasmoideo (Schizoplasmodiid Clade) – Grupo II

Este clado está formado por *Schizoplasmodium cavostelioides*, *Nematostelium ovatum* y *Ceratiomyxella tahitiensis*. Todos ellos poseen un estado trófico plasmodial y una característica estructura de unión entre la espora y el estípite, que consiste en un hilum anular en la espora que articula con una apófisis en forma de pomo situada en el ápice del estípite. Las amebas plasmodiales forman subpseudópodos filosos y subpseudópodos anastomosados, y durante su mitosis adquieren forma de vainas de guisante. *Ceratiomyxella tahitiensis* forma células ameboflageladas en su ciclo vital, y las otras dos especies, *N. ovatum* y *S. cavostelioides*, no las forman y se agrupan juntas. Todavía no se han secuenciado otros miembros del grupo como *S. obovatum*, *S. seychellarum* y *N. gracile*.

Clado Soliformovioide (Soliformoviid Clade) – Grupo III

Incluye a las dos especies del género *Soliformovum*, *S. irregulare* y *S. expulsum*, y aparece como grupo hermano de los mixomicetes. Poseen amebas flabeliformes con subseudópodos terminados en punta y con nucleolos difusos. Ambas especies forman una célula preesporal característica con aspecto de “huevo frito”.

Clado Cavostelioide (Cavosteliid Clade) – Grupo IV

En él se agrupan *Cavostelium apophysatum*, *Schizoplasmodiopsis pseudoendospora*, *Schizoplasmodiopsis amoeboidea* y *Tychosporium acutostipes*. Es el clado con morfologías más diversas. Todos poseen amebas relativamente delgadas, con subpseudópodos filosos, aunque también pueden aparecer ameboflagelados y plasmodios en los ciclos vitales de algunas especies. La mayoría muestran nucleolos centrales, excepto *S. amoeboidea*, que posee nucleolos difusos similares a los de *Soliformovum*. Sus paredes esporales están ligeramente ornamentadas, y las esporas no se desprenden del estípite.

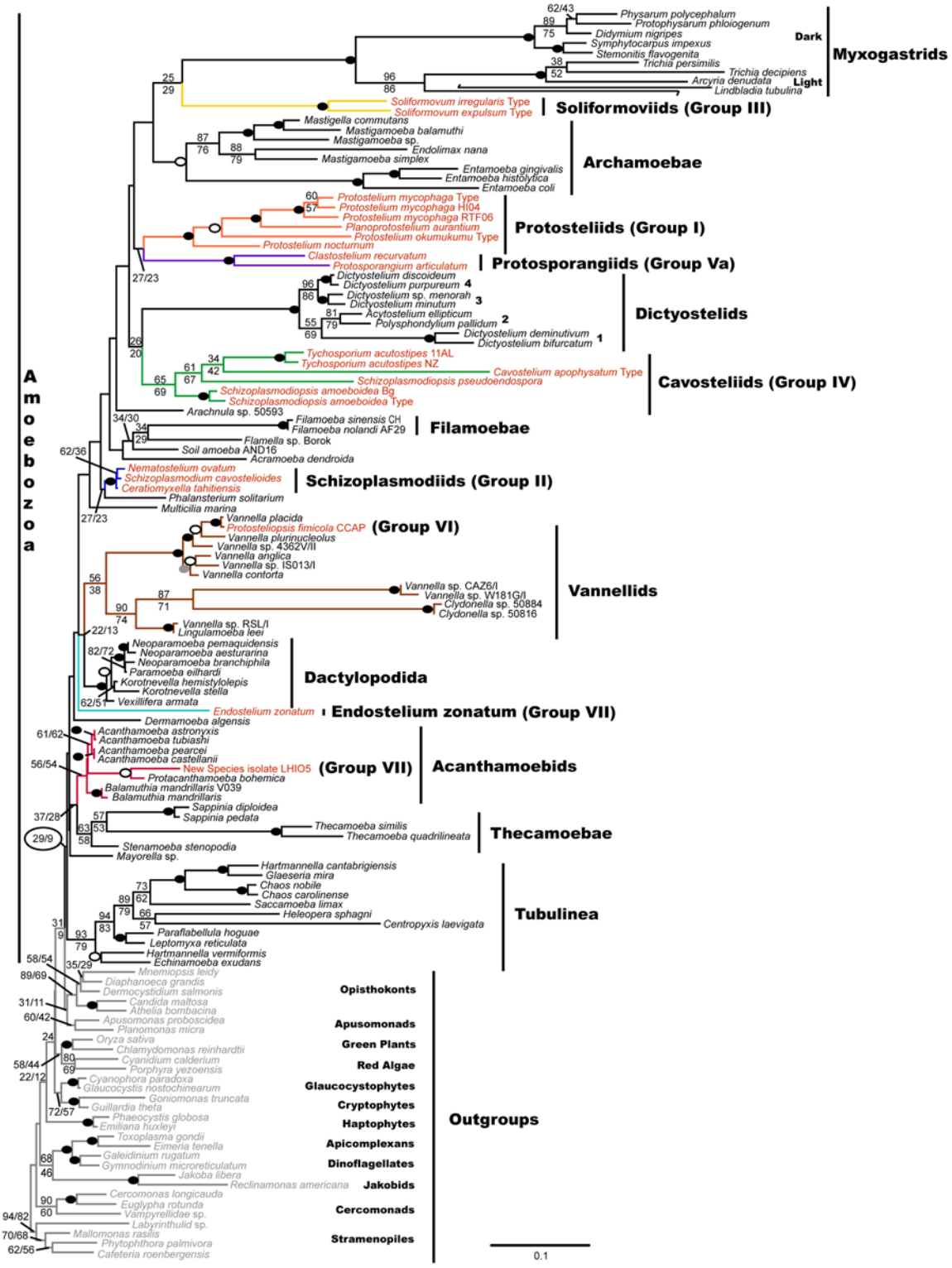


Figura 5 – Filogenia de protostélidos y otros amebozoos. Las ramas de colores destacan los linajes en los que aparecen fructificaciones de tipo protosteloide (Shadwick et at 2009a).

Clado Protosporangioide (Protosporangiid Clade) – Grupo Va en parte

Las dos especies que fueron incluidas, *Protosporangium articulatum* y *Clastostelium recurvatum*, tienen ciclos vitales idénticos, poseen ameboflagelados y amebas no flageladas similares, y cuerpos fructíferos con 2-4 esporas. Faltan por incluir el resto de las especies del género *Protosporangium*. El grupo Va de Spiegel (1990) también incluye a *Ceratiomyxa*, que posee un ciclo vital y ameboflagelados similares. Por estas razones y atendiendo a la ultraestructura de los ameboflagelados se pensaba que el grupo Va sería el grupo hermano de los mixomicetes, aunque en el trabajo de Shadwick et al (2009a) su relación no parece tan clara. El presente clado aparece como grupo hermano del clado Protosteloideo.

Grupos VI y VII

Spiegel (1990) sugirió que *Endostelium zonatum* y *Protosteliopsis fimicola* podrían ser miembros de grupos de amebas no relacionados directamente con el resto de amebas protosteloides. Los resultados de Shadwick et al (2009a) apoyan esta hipótesis. El lugar que ocupa *Endostelium zonatum* en el árbol de SSU es dudosa, pero podría pertenecer a los acantamébidos. Todo parece indicar que *Protosteliopsis fimicola* pertenece al grupo de los vannélidos, con los que comparte rasgos morfológicos, ya que posee una vacuola contráctil conspícua, velo anterior hialino, similares formas flotantes y ausencia de uropodios.

DISTRIBUCIÓN Y ECOLOGÍA

Patrones generales en microorganismos

Los organismos de pequeño tamaño, especialmente los microorganismos de vida libre, tienden a tener distribuciones más amplias y una tasa de endemismo mucho menor que las de los organismos pluricelulares, de mayor tamaño (Finlay & Clarke, 1999; Finlay et al, 1999, 2001; Finlay, 2002; Finlay & Fenchel, 2004). Se han señalado múltiples causas que podrían haber dado lugar a este patrón general. Una de ellas es que los microorganismos suelen ser localmente muy abundantes en los lugares donde habitan y, al ser tan pequeños y numerosos, aumentarían su capacidad de dispersión pudiendo fácilmente alcanzar áreas muy alejadas (Finlay, 2002). Estas características llevaron a algunos autores (Fenchel et al, 1997; Finlay, 2002) a la conclusión de que los patrones geográficos de los protistas se ajustarían a la hipótesis de Baas-Becking (1934) "todo está en todas partes y es el hábitat el que selecciona". Según esta hipótesis, en un determinado lugar podríamos encontrar todas las especies de microorganismos existentes que pueden sobrevivir con las características propias de ese hábitat en concreto (Finlay, 2002). Al contrario, en el caso de los organismos multicelulares, la existencia de barreras geográficas impide que los organismos se dispersen libremente y hace posible la especiación alopátrica. Por tanto, la diversidad local se acercaría a la diversidad global mucho más en los microorganismos que en los organismos pluricelulares (Fenchel et al, 1997; Azovsky, 2002). Al no existir ningún tipo de barrera geográfica o de factores históricos influyendo en su distribución, no habría especies de protistas endémicas, y exclusivamente serían las características del hábitat las que selecionarían las especies que viven en un determinado lugar.

Sin embargo, actualmente existen numerosas críticas a la generalización excesiva que realiza este modelo. Si bien es un hecho ampliamente aceptado que gran parte de estos organismos tienen áreas de distribución más amplias que las que suelen presentar los organismos multicelulares (Foissner, 2006), muchos autores defienden que hay al menos un cierto porcentaje de especies que se encuentran confinadas en determinado área (Smith & Wilkinson, 2007; Foissner et al, 2008; Vanormelingen et al, 2008) y aportan una hipótesis alternativa llamada "endemismo moderado". A continuación se detallan varias de las críticas realizadas a la hipótesis de "todo está en todas partes".

Déficit linneano, biodiversidad oculta y sesgo en el conocimiento de especies

La biodiversidad de organismos microscópicos es todavía muy desconocida y se estima que hay un número muy alto de especies que no han sido todavía descritas (Groombridge 1992; Foissner et al, 2002; Mora et al, 2011). Lo más probable es que las especies mejor conocidas sean precisamente los organismos más generalistas, las especies más abundantes y conspicuas que toleran un rango más amplio de variación

en su ambiente (Foissner, 2006). Por tanto, es lógico que estas especies tengan áreas de distribución amplias y se encuentren prácticamente en todas las muestras estudiadas.

También nuestro conocimiento se encuentra sesgado hacia las especies que son cultivables. La secuenciación de muestras ambientales ha permitido detectar que existe una gran diversidad de microorganismos "oculta" y que no aparece al analizar las muestas exclusivamente mediante cultivos (Ward et al, 1990). Se estima que tan solo un 1% de las especies pueden ser detectadas mediante cultivos (Whitfield, 2005). Por tanto, no es posible cuantificar ni estudiar la morfología de la inmensa mayoría de los microorganismos por estos métodos, aunque se utilicen distintos tipos de medio y diferentes condiciones. Debido a que los cultivos constituyen un filtro tan potente para la supervivencia, es probable que las especies fácilmente cultivables sean también las más generalistas y ubícuas.

Como es fácil de suponer, el estudio de muchos de estos organismos se encuentra fuertemente sesgado, habiéndose invertido más esfuerzo en el muestreo del Hemisferio Norte, especialmente en Europa y Norte América, que en el estudio de muchas otras áreas del mundo. Para muchos grupos de organismos existen grandes áreas vacías en las que nunca se ha investigado y sobre las que no se tiene ningún tipo de información. Como consecuencia, la falta de datos hace que no se conozca en detalle la distribución global de prácticamente ninguna especie.

Dificultades en la identificación y especies estandarte

Comparados con los organismos multicelulares, los organismos microscópicos suelen presentar pocos caracteres morfológicos. En muchas ocasiones para poder identificar las especies con fiabilidad es necesario el uso de microscopía electrónica. La taxonomía de estas especies es especialmente complicada y requiere de mucha experiencia por parte del investigador que la realiza. A esto se suma que hay pocos profesionales dedicados a este tipo de trabajo (Foissner, 2009). Esto produce una gran escasez de citas y una mayor frecuencia de errores en la determinación de las especies (Foissner 2006, 2009), lo que añade ruido y dificulta los análisis biogegráficos y ecológicos.

Es difícil distinguir las especies que tienen áreas de distribución restringidas de las que no han sido adecuadamente muestreadas o las que han sido mal identificadas con gran frecuencia. Las especies estandarte son aquellas que presentan una morfología muy característica, llamativa y fácilmente detectable, por lo que es difícil que se pasen por alto en los estudios o que sean confundidas con otros organismos. Estas especies presentan la ventaja de que los datos existentes sobre ellas son mucho más fiables. Los ejemplos de especies estandarte con áreas de distribución limitadas, como *Nebela vas* Certes (Smith & Wilkinson, 2007) son especialmente valiosos, porque no estarán afectados por las fuentes de error a las que nos hemos referido.

Existencia de criptoespecies y el concepto de especie

Los protistas han surgido hace muchos millones de años y tienen tiempos generacionales muy cortos. La conjunción de ambos factores ofrece muchas posibilidades para la especiación (Foissner, 2006, 2009). A pesar de ello, se ha calculado que tienen

una diversidad global muy baja comparada con la de los organismos multicelulares (Finlay, 2002). Una posible explicación es que la enorme capacidad de dispersión de los protistas haría que que fuesen capaces de superar cualquier barrera geográfica para la migración, por lo que su tasa de especiación alopátrica sería muy baja (Finlay & Fenchel, 2004). Sin embargo Foissner (2006, 2009) afirma que la aparente escasez global de especies se debe a la gran cantidad de biodiversidad que queda por describir debido a un muestreo insuficiente.

El reciente descubrimiento de la existencia de complejos de criptoespecies de protistas (Amato et al, 2007; Smirnov, 2007; Morard et al, 2009; Douglas et al, 2011) hace necesario tener en cuenta este fenómeno en el estudio su biogeografía. Estos complejos están constituidos por grupos de organismos con la misma morfología pero con claras diferencias genéticas, y que son en realidad varias especies muy cercanas entre si. Al estudiar la distribución de un organismo teniendo en cuenta exclusivamente caracteres morfológicos es posible que en realidad se estén considerando como una misma especie varias especies distintas, que incluso tengan diferentes características ecofisiológicas o diferentes distribuciones. Muchas veces la frontera que marca la diferencia entre dos especies y dos ecotipos no es una línea clara (Weise, 2006), especialmente en organismos que no presentan reproducción sexual. La falta de consenso sobre la definición de lo que es una especie en protistas también dificulta dar una respuesta al debate sobre su biogeografía (Mitchell & Meisterfeld, 2005).

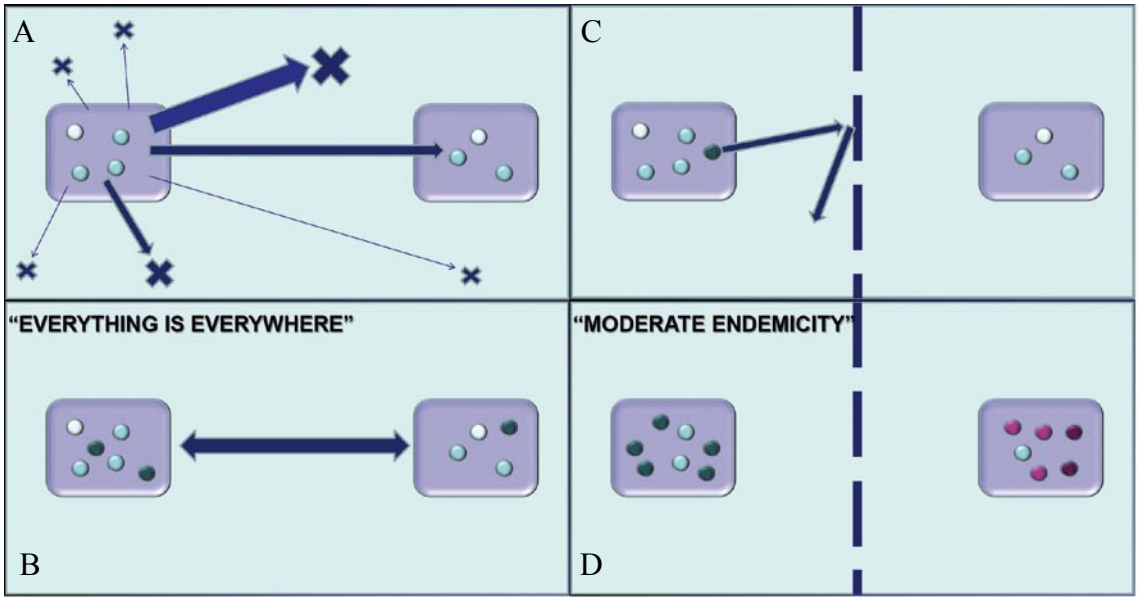


Figura 6 – Hipótesis sobre patrones biogeográficos en protistas. A, B: "Todo está en todas partes", C, D: "Endemismo moderado", A: La dispersión se produce en todas las direcciones, pero son las características del hábitat alcanzado las que permiten o no la supervivencia de los organismos, B: En un determinado hábitat aparecerán siempre los mismos organismos, sin importar la localización geográfica, C: La existencia de barreras geográficas impide la dispersión de los organismos, D: En un mismo tipo de hábitat a ambos lados de una barrera geográfica se encuentran distintos organismos.

El factor tiempo

En el estudio de los efectos de la dispersión de los microorganismos, es importante tener en cuenta no sólo la dimensión espacial, sino también la temporal. El acervo genético de una especie, que en un principio puede ser común en todo su rango geográfico, con el tiempo puede verse fragmentado y aislado debido a que las poblaciones desarrollen adaptación local (Medlin, 2007). Al realizar un estudio filogeográfico en realidad estamos estudiando una "instantánea" de un proceso dinámico, por lo que en cada caso se podrá apreciar una distinta etapa en el proceso de expansión de una nueva mutación o de una nueva especie (Medlin, 2007).

También con el paso del tiempo se pueden ir acumulando diferencias entre ecotipos. Cuando aparece una mutación beneficiosa en bacterias, la fuerte competencia puede llegar a extinguir el resto de cepas del mismo ecotipo. La extinción generalmente no afectará a cepas de otros ecotipos porque no compiten por los mismos recursos. Este proceso de divergencia adaptativa, mediante sucesivas eliminaciones de la diversidad interna se conoce como selección periódica (Atwood et al, 1951), y también podría estar presente en protistas (Finlay, 2004).

Otra cuestión a tener en cuenta es la dinámica de los procesos de dispersión. Se desconoce si existen vías preferentes para la dispersión o si ésta se realiza con equiprobabilidad en todas las direcciones. También aspectos como el tiempo necesario para que una nueva mutación surgida alcance nuevas zonas están todavía por investigar.

Influencia de la ecología

La ecología de microorganismos es un campo que se encuentra actualmente en expansión, y todavía su base teórica no está completamente desarrollada (Prosser et al, 2007). Al tener estos organismos una dispersión muy eficaz, el estudio de su ecología cobra especial importancia, puesto que actúa como principal filtro para el establecimiento y supervivencia de los propágulos. El hábitat selecciona entre los múltiples propágulos que acceden a él qué organismos son capaces de sobrevivir con el conjunto de características que posee (Finlay, 2002).

Muchos microhábitats constituyen verdaderas islas ecológicas para la supervivencia de determinados organismos. Saltar de una isla ambiental propicia a otra puede requerir atravesar largas distancias a través de zonas desfavorables. Siguiendo las ideas de MacArthur & Wilson (1967), es posible que los organismos especialistas que sobreviven exclusivamente en determinados tipos de ambientes pequeños, discontinuos, y alejados ente sí puedan tener una probabilidad mucho menor de alcanzar ambientes apropiados cuando se dispersan que los organismos más generalistas que vivan en hábitats más continuos y menos parcheados. Además, el tipo de hábitat en el que los organismos viven y su accesibilidad pueden tener mucha influencia en su capacidad de dispersarse. Es de suponer que es más probable que sean transportados los organismos del plancton marino que los que viven en las capas inferiores del suelo (Mitchell & Meisterfeld, 2005). Las probabilidades de alcanzar un ambiente adecuado estarán influenciadas también por la resistencia de los propágulos y por su capacidad para ser viables tras largos periodos de dormancia (Hughes Martiny et

al, 2006). Esta capacidad puede ser muy variable. Por ejemplo, los quistes de ciliados y flagelados formados en hábitats con condiciones más extremas resisten la sequedad y el frío durante largos periodos de tiempo (Foissner, 1996; Foissner et al, 2002), mientras que los que son producidos en el suelo de los bosques tropicales sólo sobreviven unos pocos meses (Foissner, 1997).

En resumen, el estudio de la biogeografía de protistas está condicionado por la necesidad de avances tanto conceptuales como metodológicos. El estudio de los mecanismos dispersivos y ecológicos implicados es básico para poder llegar a comprender los procesos que delimitan la distribución de las especies y está considerado como un reto para el futuro. Desde el punto de vista metodológido, el uso de caracteres moleculares se perfila como una herramienta eficaz, pues permite recopilar citas corológicas con mayor fiabilidad y estudiar la variabilidad que existe dentro de una misma morfoespecie. Pese a que las distribuciones amplias parezcan ser el patrón general, este modelo de distribución no es común para todos los organismos. No obstante, el gran desconocimiento que poseemos de estos organismos hace que todavía falte mucho para entender en profundidad su biogeografía.

Distribución y ecología de Eumycetozoa

El estudio de la ecología y distribución de los protostélidos y mixomicetes está afectado por el marco general anteriormente descrito, además de por las particularidades de estos organismos que pasamos a detallar a continuación.

Protostélidos

Los estudios realizados sobre amebas protosteloides son muy escasos, por lo que se hace muy complicado esbozar en líneas generales su distribución y preferencias ecológicas. Hasta hace muy poco los investigadores se centraban exclusivamente en la descripción detallada de la morfología de las nuevas especies descritas, sin prestar apenas atención al lugar o las condiciones en que dichas especies habían sido descubiertas. La mayoría de las especies descritas han sido encontradas sobre tejidos vegetales muertos. Este sustrato se divide habitualmente en dos microhábitats para su estudio: uno de ellos, la hojarasca del suelo, está formado por el conjunto de palitos, hojas y otros restos vegetales que se extienden sobre la superficie del suelo, y el otro, la hojarasca aérea, por los tejidos muertos que todavía permanecen adheridos a las plantas, como por ejemplo hojas secas que todavía no han caído al suelo. La corteza de plantas vivas también es un sustrato en el que aparecen amebas protosteloides con gran frecuencia, pero lamentablemente las especies que crecen sobre corteza son muy difíciles de aislar para su estudio (Olive, 1975).

Fueron los trabajos de Spiegel y Moore (Best & Spiegel 1984; Moore & Spiegel 1995, 2000a,b,c) los primeros en ofrecer listados de especies encontradas en distintas localidades, y los primeros también en describir las diferencias en las comunidades de protostélidos encontradas en distintos microhábitats en una misma localidad. En una etapa posterior se intentaron ampliar los datos disponibles mediante el estudio de zonas muy alejadas entre si y con diferentes climas. Se han realizado estudios ecológicos sobre estos organismos en Norte América (Moore & Spiegel, 1995, 2000

b, c; Lindley et al, 2007; Brown & Spiegel, 2008; Shadwick et al, 2009b), en el Neotrópico (Stephenson et al, 1999; Moore & Spiegel, 2000a; Moore & Stephenson, 2003; Stephenson et al, 2004), en Europa (Tesmer et al, 2005, 2009), en Australia (Powers & Stephenson, 2006), en África (Ndiritu et al, 2009a), en India (Shadwick & Stephenson, 2004), así como en bosques boreales y tundra de Alaska (Moore et al, 2000), Siberia (Kosheleva et al, 2009) y la isla de Macquarie (Spiegel & Stephenson, 2000).

Parece que al menos la mayor parte de ellos son organismos con amplia distribución y capaces de vivir desde en zonas tropicales hasta en zonas boreales (Spiegel, 1990), pero la composición y la estructura de las comunidades de prototélidos en cada microhábitat varía entre zonas con distintos climas. La tendencia general es que las especies que son propias de microhábitats alejados del suelo en zonas templadas se transladen a él en las localidades con mayor elevación y mayor latitud, mientras que las especies que viven preferentemente sobre la hojarasca del suelo en zonas templadas prefieran ambientes más alejados del suelo en las localidades tropicales (Moore & Spiegel, 2000a; Spiegel & Stephenson, 2000).

Myxomycetes

Los datos existentes sobre mixomicetes son mucho más abundantes aunque irregularmente repartidos. Lamentablemente hay pocos trabajos publicados que indaguen sobre sus patrones geográficos (Stephenson et al, 2008), y la investigación de su ecología se encuentra en sus estados iniciales. Su tradicional estudio enmarcado en el campo de la micología ha permitido que enfoques propios de esta ciencia fueran aplicados a estos organismos, y que haya existido un mayor interés por generar información en forma de citas corológicas. El almacenamiento de sus cuerpos fructíferos en herbarios, permitiendo conservar el material recolectado, hace posible acceder a él con mayor facilidad, y que se puedan revisar las identificaciones y elaborar listados de citas con mucha mayor fiabilidad que en la mayoría de grupos de protistas.

Los mixomicetes han sido estudiados más frecuentemente desde un punto de vista taxonómico, haciendo hincapié en aspectos relacionados con la catalogación y la descripción de nuevos taxones, por lo que los estudios ecológicos son mucho menos frecuentes y, en su mayor parte, han sido realizados en las últimas décadas (Maimoni-Rodella & Gottsberger, 1980; Eliasson, 1981; Stephenson, 1988). Por esta razón todavía faltan muchos datos sobre la naturaleza de las interacciones que existen entre estos organismos y su ambiente. Los datos disponibles están fuertemente sesgados hacia ciertas áreas de muestreo. La mayoría de los estudios sobre mixomicetes se han realizado en bosques templados del Hemisferio Norte (Stephenson, 2011), principalmente Europa y Norte América. En las últimas décadas se han comenzado a explorar otras áreas como Nueva Zelanda (Stephenson, 2003), Australia (Black et al, 2004), Asia (Takahashi, 2004; Novozhilov & Schnittler, 2008a), la región neotropical (Lado & Wrigley de Basanta, 2008), y África (Ndiritu et al, 2009b), pero todavía hay muchas zonas cuya diversidad de mixomicetes es totalmente desconocida. También relativamente reciente es el estudio de cierto tipo de ambientes como desiertos (Blackwell & Gilbertson, 1980; Schnittler, 2001a; Novozhilov et al, 2006; Lado et al, 2007), bosques tropicales (Alexopoulos, 1970; Farr, 1976; Stephenson et al, 1998;

Schnittler and Stephenson, 2000; Lado et al, 2003), bosques boreales y tundra (Stephenson & Laursen, 1993, 1998; Stephenson et al, 2000; Kosheleva et al, 2008), zonas insulares (Eliasson, 2004; Stephenson et al, 2007; Rojas & Stephenson, 2008), y zonas de montaña (Lado, 2004; Lado et al, 2005; Rojas & Stephenson, 2007; Novozhilov & Schnittler, 2008b; Ronikier & Ronikier, 2009; Stephenson & Shadwick, 2009), que han aportado nueva información. También han aparecido mixomicetes en estudios de ecosistemas acuáticos (Lindley et al, 2007).

Los datos existentes parecen señalar que la mayor parte de los mixomicetes posee distribuciones muy amplias y pueden aparecer en la mayor parte de los ecosistemas terrestres (Stephenson, 2011), aunque también se conocen numerosas especies descritas que han aparecido en muy pocas localidades, por lo que es muy probable que tengan áreas de distribución restringidas. En varios estudios realizados en islas oceánicas como Macquarie (Stephenson et al, 2007) y las islas Hawaii (Eliasson, 1991), aparecen una gran variedad de especies, lo que parece indicar que las esporas de dichas especies pudieron ser transportadas a muy larga distancia desde los continentes. El patrón general latitudinal muestra que la diversidad de estos organismos es mayor en las zonas templadas que en las zonas tropicales o boreales (Schnittler, 2001b), aunque la diferencia en el esfuerzo de muestreo empleado en las distintas zonas, hace que este patrón sea muy cuestionable (Rojas, 2010).

Estudios recientes (Stephenson et al, 1993, 2008) señalan que a primera vista la distribución de muchos de estos organismos está principalmente relacionada con ciertos factores ambientales, destacando:

- Las diferencias en el clima y/o la vegetación a escala global.
- Las diferencias en los microhábitats particulares a escala local.

Los mixomicetes están asociados con un gran número de microhábitats distintos. Se pueden encontrar creciendo sobre madera en descomposición, hojarasca, en el suelo, excrementos de herbívoros y corteza de plantas vivas, y recientemente se están investigando nuevos sustratos como tejidos de plantas suculentas (Mosquera et al, 1999) inflorescencias (Schnittler & Stephenson, 2002), briófitos (Schnittler, 2001c), y lianas (Wrigley de Basanta et al, 2008). Cada uno de estos microhábitats se caracteriza por poseer un conjunto más o menos distintivo de especies, y ciertos grupos de mixomicetes tienden a aparecer asociados con determinadas especies vegetales. Se conocen mejor los mixomicetes que viven sobre restos de madera, debido a que suelen ser especies con un tamaño suficiente como para ser detectadas a simple vista en el campo (Martin & Alexopoulos, 1969). Los mixomicetes asociados a la corteza de plantas vivas o la hojarasca son mucho menos conocidos, pues por su pequeño tamaño o su aparición esporádica no pueden ser fácilmente detectados en el campo. Sin embargo, el cultivo en cámara húmeda (Gilbert and Martin 1933) ha permitido una mayor capacidad de detección de este tipo de especies.

Como en el caso de los protostélidos, las comunidades de mixomicetes que viven en diferentes microhábitats en una misma localidad pueden ser muy diferentes entre sí, y estas comunidades varían a su vez en las distintas zonas estudiadas según el clima. La temperatura y la humedad parecen ser los principales factores limitantes para la

supervivencia de los mixomicetes en la naturaleza (Alexopoulos, 1963), y la riqueza de especies a nivel local tiende a aumentar cuando aumenta la diversidad y la biomasa de plantas vasculares. La diversidad de plantas produciría un mayor número de nichos diferentes para ser ocupados por distintas especies de bacterias y otros organismos descomponedores de los que los mixomicetes se alimentan (Madelin, 1984; Stephenson, 1989). En bosques tropicales, la biodiversidad de mixomicetes parece ser mayor en los microhábitats alejados del suelo y alejados de la humedad, mientras que en áreas templadas y boreales, la biodiversidad es mayor en microhábitats del suelo y en las zonas húmedas (Stephenson, 2011). Otro factor que se ha señalado como de gran importancia para los mixomicetes es el pH de los sustratos (Harkönen, 1977; Stephenson, 1989; Wrigley de Basanta, 2000).

El uso de técnicas moleculares para el estudio de la ecología de mixomicetes, como la secuenciación de muestras ambientales, es todavía muy escaso (Win Ko Ko et al, 2009; Kamono & Fukui, 2006; Kamono et al, 2009a b), pero es de esperar que en un futuro próximo y debido al potencial de estas técnicas para generar información, su aplicación se generalice. También el uso de caracteres moleculares permitirá determinar la existencia de complejos de especies crípticas y cómo se distribuyen, ya que por otros métodos ya conocemos que muchas morfoespecies consisten en complejos formados por diferentes cepas con distribución geográfica restringida y que no se reproducen sexualmente entre sí (El Hage et al, 2000; Clark, 2000; Clark and Stephenson, 2000; Irawan et al, 2000; Fiore-Donno et al, 2011). Estas líneas aisladas genéticamente pueden ser capaces de evolucionar independientemente, acumulando mutaciones de manera diferente y adaptándose a un conjunto particular de condiciones ambientales (Stephenson el al, 2008).

Objetivos

A continuación se detallan los objetivos y las hipótesis en los que se ha basado el trabajo presentado en esta memoria.

Objetivo general

El objetivo general de esta memoria es estudiar la influencia de factores ambientales (climáticos y microhábitat) sobre la distribución geográfica de varias especies de eumicetozoos. El presente trabajo se centra en dos grupos de organismos con distintas características: los protostélidos y los mixomicetes.

Como ha sido explicado anteriormente, los organismos de pequeño tamaño típicamente tienen distribuciones amplias y una baja tasa de endemismo (Finlay, 2002, 2004; Finlay & Fenchel, 2004), pero la existencia de excepciones probadas (Smith & Wilkinson 2007) y el hecho de que los datos disponibles sean escasos y estén fuertemente sesgados (Foissner, 2006), hace que todavía falte mucho para entender con detalle cómo son sus patrones geográficos y cuáles son los mecanismos dispersivos y ecológicos implicados en darles forma.

Hipótesis de trabajo

El trabajo presentado en esta memoria se puede enmarcar fundamentalmente en dos hipótesis:

• La primera hipótesis es que la alta capacidad dispersiva de estos organismos hace que las características del ambiente cobren especial importancia en la distribución de los Eumycetozoa, y que diferentes organismos muestren distintas preferencias.

Para responder a estas preguntas se estudió la distribución de las amebas protosteloides en la Península Ibérica. Los protostélidos son los eumicetozoos que han sido más recientemente descubiertos (Olive & Stoianovitch, 1960) y los más desconocidos. Al poseer fructificaciones de pequeño tamaño, para su estudio es siempre necesario realizar cultivos de los sustratos recolectados y usar un microscopio. Hasta la fecha muy pocas zonas del mundo han sido muestreadas para estos organismos y apenas existen conocimientos sobre su distribución geográfica ni sobre sus preferencias ecológicas. Cuando se inició el trabajo en esta tesis doctoral, los protostélidos no habían sido nunca estudiados en detalle en ninguna zona de Europa, de donde sólo existían algunas citas aisladas (Olive, 1962, 1967, 1975; Glustchenko et al, 2002; Tesmer et al, 2005). Tampoco se disponía de ningún dato sobre la presencia de estos organismos en ninguna de las cinco zonas del mundo con clima mediterráneo. Los trabajos sobre la ecología del grupo que han sido publicados se han centrado en la comparación de las especies presentes en distintos sustratos a escala local. Estudiar

los protostélidos a escala Ibérica con una metodología uniforme hace posible evaluar y comparar los efectos de distintas variables climáticas y de los microhábitats.

• La segunda hipótesis de partida es que, al menos en ciertos casos, pueden encontrarse cepas con distribución limitada, en las que la dispersión de los propágulos no se realiza de forma instantánea y equiprobable, sino que se produce más fácilmente en ciertas direcciones.

Esta parte del trabajo se realizó mediante el estudio del myxomycete *Badhamia melanospora* Speg. Los mixomicetes son los eumicetozoos con cuerpos fructíferos de mayor tamaño, que en muchos casos pueden ser observados a simple vista en el campo. El material recolectado puede conservarse en herbarios permitiendo el estudio posterior de las muestras. En contraste con los protostélidos, existe una mayor cantidad de información previa disponible, tanto sobre su morfología, como sobre su ecología, sus ciclos vitales y sus sistemas reproductivos. También están disponibles un mayor número de citas corológicas a escala global (GBIF, www.gbif.org), aunque todavía existen amplias áreas en el mundo que aún no han sido muestreadas para estos organismos, especialmente en Sudamérica, África y Asia.

Objetivos particulares

Para abordar el objetivo general mediante el estudio de las dos hipótesis planteadas fue necesario establecer una serie de objetivos particulares que a continuación se detallan.

1. Caracterizar la diversidad de morfoespecies de amebas protosteloides a escala ibérica y averiguar cómo influyen diferentes factores ambientales sobre sus abundancias en las localidades estudiadas. Para ello ha sido necesario:

• Comprobar la presencia de los protostélidos en las zonas con clima mediterráneo del suroeste de Europa
• Optimizar el método de muestreo y de cultivo, no sólo para ajustar el esfuerzo a las particularidades de esta zona mediterránea, sino también para proporcionar un método cuantitativo y estadístico que permita la comparación entre ecosistemas diferentes a mayor escala de la habitual.
• Elaborar un listado comentado de las especies presentes en la península Ibérica. Para ello se han muestreado 97 localidades, recogiendo muestras de tres microhábitats diferentes: hojarasca del suelo, hojarasca adherida a las plantas y corteza de plantas vivas.
• Observar las diferencias en composición de especies y sus abundancias entre las localidades estudiadas y los distintos microhábitats.
• Evaluar la influencia de diferentes factores climáticos y del tipo de microhábitat en la abundancia de las especies utilizando estadística multivariante.
• Realizar modelos de nicho ambiental, en los casos en los que el número de citas lo permita, para identificar las zonas con mayor probabilidad de presencia de protostélidos en la Península Ibérica.

2. Estudiar la variabilidad existente en una morfoespecie de myxomycete (*Badhamia melanospora*) a nivel global en un contexto filogeográfico, para detectar los patrones de distribución de las distintas cepas, observar su morfología en detalle, ver si tienen diferentes prefe-

rencias ecológicas, y valorar si la dispersión ha estado limitada por la influencia de barreras geográficas o factores históricos.

• Realizar un análisis de la variabilidad intraespecífica de un fragmento del ADN que codifica para la subunidad pequeña del ribosoma (SSUr ADN). Para ello se ha muestredo en el rango geográfico completo conocido de la especie, y se han seleccionado los especímenes para obtener una muestra final lo más representativa posible.
• Hacer un estudio más preciso de la morfología de la espora utilizando microscopia electrónica de barrido (SEM), para comparar los resultados con la genealogía anteriormente obtenida.
• Explorar la distribución geográfica de las variantes para una mejor comprensión de la historia evolutiva de la especie.
• Como se ha encontrado un patrón geográfico claro, otro objetivo ha sido testar la hipótesis alternativa de que estén operando adaptaciones específicas de cada clado a las condiciones medioambientales distintas en cada zona mediante la comparación de modelos de nicho ecológico.

Capítulo 1: Presencia de Protostélidos en la Península Ibérica

Con anterioridad al inicio de este trabajo, apenas se disponía de datos sobre la presencia de protostélidos en Europa, y no había ninguna cita de estos organismos en la Península Ibérica. Por tanto, cabía la posibilidad de que gran parte de los protostélidos no fueran capaces de sobrevivir en zonas con este tipo de clima o que no pudieran crecer sobre la vegetación autóctona de la Península.

Tampoco se disponía apenas de información sobre la ecología de las especies, y los estudios hasta entonces publicados se habían realizado casi siempre a escala local y en zonas con otro tipo de climas. Debido a la falta de datos, en ese momento era muy complicado poder diseñar un muestreo para caracterizar la ecología de las especies en un área de estudio de mayor tamaño como es la península Ibérica, sobre la que además no se disponía de ninguna información previa.

Por tanto, fue necesario realizar un primer muestreo en una zona pequeña como paso previo. De esta manera se pudo aprender a seleccionar los sustratos más apropiados en el campo, a preparar los medios de cultivo para estos organismos, a realizar dichos cultivos y a identificar y aislar las especies encontradas, adquiriendo cierta experiencia que era necesaria para poder plantear el resto del trabajo. También mediante la realización de este estudio fue posible observar cómo se distribuían las especies en los diferentes microhábitats, lo que comparado con la información sobre la ecología de las especies en otras zonas del mundo, permitió establecer las primeras hipótesis de trabajo.

Para ello se escogió realizar un listado de las especies encontradas en en el Parque Natural de Somiedo (Asturias), puesto que se trata de una zona bien conservada, con un clima en transición entre el eurosiberiano y el mediterráneo, y con un relieve acusado que permitió muestrear en zonas con diferente altitud. Los resultados de esta primera incursión en el estudio de los protostélidos ibéricos se publicaron en el artículo que se incluye a continuación:

Aguilar, M., Lado, C. & Spiegel, F. W. (2007) Protostelids from deciduous forests: first data from southwestern Europe. Mycological research 111(7):863-872.

Resumen: En este artículo se presentan los primeros datos sobre protostélidos del suroeste de Europa. Se identificaron un total de 21 especies en muestras recolectadas en la Reserva de la Biosfera de Somiedo (España). Ésta es la mayor riqueza de especies registrada hasta el momento en Europa o en una latitud tan alta (<40º). Siete de las especies (*Cavostelium apophysatum*, *En-*

dostelium zonatum, *Microglomus paxillus*, *Protosporangium fragile*, *Protostelium okumukumu*, *Soliformovum expulsum* y *Schizoplasmodiopsis micropunctata*) son nuevos registros para Europa. En esta reserva se han econtrado aproximadamente el 65% de las especies de protostélidos microscópicos descritos en el mundo, un hecho que aumenta el valor biológico de esta zona protegida y sugiere que España es un excelente lugar para estudiar el grupo. Se ha realizado un estudio de los microhábitats, encontrando dfierencias en la composición y abundancia de especies entre los sustratos hojarasca del suelo, la hojarasca aérea y la corteza. Se incluyen comentarios sobre la distribución y ecología de las especies, así como ilustraciones de algunas de las especies.

MYCOLOGICAL RESEARCH 111 (2007) 863–872

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Protostelids from deciduous forests: first data from southwestern Europe

María AGUILAR[a,*], *Carlos LADO*[a], *Frederick W. SPIEGEL*[b]

[a]*Departamento de Micología, Real Jardín Botánico, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain*
[b]*Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA*

ARTICLE INFO

Article history:
Received 12 December 2006
Received in revised form
30 March 2007
Accepted 24 May 2007
Published online 3 June 2007
Corresponding Editor: John Dighton

Keywords:
Eumycetozoans
Inventory
Protists
Slime moulds
Spain

ABSTRACT

The first data of Protostelids from the southwest of Europe are presented in this paper. A total of 21 species were identified from samples collected in Somiedo Biosphere Reserve (Spain). This is the highest species richness recorded to date for Europe or for a latitude this high (>40°). Seven species (*Cavostelium apophysatum*, *Endostelium zonatum*, *Microglomus paxillus*, *Protosporangium fragile*, *Protostelium okumukumu*, *Soliformovum expulsum* and *Schizoplasmodiopsis micropunctata*) are new records for Europe. Approximately 65 % of the microscopic protostelid species described in the world have been found in this Reserve, a fact that increases the biological value of this protected area and suggests that Spain is an excellent location to study this group. A microhabitat study has been carried out finding differences in species composition and abundance between ground litter, aerial litter, and bark substrates. Comments on the distribution and ecology of the species, as well as illustrations of some species are included.

Introduction

Protostelids, a widespread group of simple mycetozoans (*Eumycetozoa*, *Amoebozoa*; Adl *et al.* 2005) producing microscopic fruiting bodies usually bearing a single spore at the tip of a delicate stalk, can be readily isolated from a great variety of substrates such as dead attached plant parts, soil, humus, dung, or bark (Olive 1975a). The first species of the group was found only 45 y ago by Olive & Stoianovitch (1960), who incidentally isolated *Protostelium mycophaga* on dead florets of *Phragmites australis* from Somerville (New Jersey), as they were attempting to culture *Acrasis rosea*. Since then, more than 30 species of protostelids have been described by studying material from several parts of the world (Spiegel *et al.* 2005; Hernández & Lado: *An on-line nomenclatural information system of Eumycetozoa*; http://www.nomen.eumycetozoa.com).

It is remarkable that Europe, one of the most studied territories of the world in terms of biodiversity, has hardly been surveyed for this group. The published works that contain European records are few: those carried out by Olive (1962, 1967, 1975b) more than 30 y ago, two records from Ukraine (Glustchenko *et al.* 2002), and a recent survey from beech forests of Germany (Tesmer *et al.* 2005). No studies have taken place in the southwest of Europe; this being the first study of protostelids made in this part of the world.

Information relating to ecology and distribution of the group has increased recently, but still relatively little is known. The data that are available would seem to indicate

* Corresponding author.
E-mail address: aguilar@rjb.csic.es
0953-7562/$ – see front matter © 2007 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.mycres.2007.05.010

that compositional differences exist for the assemblages of species associated with particular types of microhabitats (Moore & Spiegel 1995, 2000a,b,c; Stephenson & Moore 1998).

The purpose of this paper is to establish a biodiversity survey of the protostelid species present in the southwest of Europe and to report their relationship to their environmental factors in order to guide further studies.

Materials and methods

Study area

This study is based on material collected in October 2005 in Somiedo Biosphere Reserve, which is located in the northwest of Spain, in the province of Asturias, between 43°00′- 43°11′N and 6°04′- 6°22′W. The entire 29 100 ha Reserve is located on the northern slope of the Cordillera Cantábrica, in a range of elevation between 395 m and 2194 m. The landscape is dominated by mountains, U-shaped valleys and glacial lakes, and the lithology is varied and peculiar, with alternating siliceous and calcareous formations. The climate is oceanic, with frequent fog, high humidity, rain and snow, having an annual medium precipitation of 1030–1350 mm; and the temperature (mean annual temperature 8 °C) is regulated by influence of the Cantabrian sea. In addition, the altitude causes a certain degree of continentality in the climate, so this area has transitional characteristics between the temperate climate of the northern areas of Spain, and the greater extremes of the central plain where the climate becomes Mediterranean. The predominant vegetation in the study area is boreal forests (mixed broadleaf forests with oaks, beeches, chestnut, and hazelnut), shrublands, and grasslands. This area has high human influence but most is based in sustainable uses as traditional agriculture and stockbreeding.

Sampling

A total of 12 localities (Table 1) was sampled. All samples were segregated according to microhabitat during the sampling by placing them in different paper bags and air drying. Then they were sent to the laboratory of the Real Jardín Botánico and designated as collection AS05 (AS for Asturias). Results in previous studies suggest the protostelid biota differs according to microhabitat in temperate regions (Moore & Spiegel 2000a,b,c; Best & Spiegel 1984). The samples were collected from three different microhabitats: bark from living trees, ground litter, and aerial litter. The ground litter microhabitat was defined as the layer of twigs, leaves, and other plant debris extending over the soil surface, whereas the aerial litter microhabitat was defined as the assemblage of dead but still attached parts of standing plants.

As a preliminary study, 68 samples were randomly selected from the 121 collected samples. These samples included, 30 from ground litter, 32 from aerial litter, and six from bark. Primary isolation plates were prepared between October 2005 and March 2006, using a modification of the technique described by Olive (1975a); (see also Moore & Spiegel 1995 and Spiegel *et al.* 2005). One plate per sample was prepared as follows: the material was cut into small (*ca* 1.5–2 cm) pieces with sterile forceps and then soaked in sterile water. Eight pieces from each sample were plated out forming a circle on a 9 cm Petri dish with a weak nutrient medium (wMY: 0.002 g malt extract, 0.002 g yeast extract, 0.75 g K_2HPO_4, 15 g agar l^{-1} of distilled water). The plates were incubated at ambient laboratory temperature (20–24 °C) and were surveyed for protostelids in the second week of culture. Species were identified on the basis of fruiting body morphology under the light microscope using the ×10 objective. When necessary and possible, fruiting bodies were also examined with ×20 objective to help confirm the identification. Isolations to culture were made, if necessary, to confirm the constancy of characters. Photomicrographs (Figs 1–2) were taken with a Nikon Eclipse E600 compound microscope using bright-field optics and a Nikon Digital Sight DS-5M digital camera.

Occurrences of species that were observed were recorded simply as present on a given sample of substrate (number of identifications). Although a species may have occurred in many patches in some samples and only once in others, we were interested in a simple survey of the protostelid biota, and did not design the survey to collect more detailed quantitative data.

Nomenclature used herein follows Olive (1975a) and Hernández & Lado www.nomen.mycetozoan.com. Identifications were made using both Spiegel *et al.* (2005) and original descriptions.

Data analysis

To estimate the extent to which the survey was exhaustive in terms of recorded species, a species accumulation curve was constructed (Schnittler 2001; Schnittler & Stephenson 2000). The sequence of samples was randomly permutated 100 times and the means of the cumulated number of species were calculated with a program developed in the laboratory of Real Jardín Botánico. The plot of the mean cumulated number of species *versus* the number of samples was subjected to a regression analysis, using the saturation formula

$$y = Ax/(B + x)$$

where x is the number of samples, y represents the number of species recorded, and the parameter A refers to the maximum number of species to be expected and B is the number of samples needed to reach half of the number of species to be expected.

Results

Ecology

A total of 164 occurrences, incorporating 21 species of protostelids, were recorded in this study. An estimate of 25 species ($A = 25$) to be expected was obtained from the BS analysis (Fig 3). Comparing the actual number of species with this estimation, the survey was complete to 84.2 %. Considering the different microhabitats (Fig 4) the survey was complete to 78.7 % ($A = 17.8$) for ground litter, and 73.2 % ($A = 21.9$) for aerial litter. Bark samples did not give a reasonable fit. It can be assumed that our sampling effort was sufficient for recovering all of

Table 1 – Sampled localities, their characteristics, and the code for samples deposited in the Departamento de Micología, Real Jardín Botánico

	Locality	Coordinates	Altitude	Sampling date	Vegetation	Simples
Loc. 1	Spain, Asturias, Teverga, Vigidel	43.14636° N 06.14100° W	630 m	4 Dec. 2005	Mixed forest with *Castanea sativa*, *Acer* sp., *Fagus sylvatica*	AS05-1 – AS05-12
Loc. 2	Spain, Asturias, Teverga, Puerto de San Lorenzo	43.14056° N 06.19333° W	1310 m	4 Dec. 2005	*Ilex aquifolia* forest and mountain grassland	AS05-13 – AS05-26
Loc. 3	Spain, Asturias, Somiedo, Las Viñas	43.15278° N 06.26472° W	740 m	4 Dec. 2005	Path with *Corylus avellana*, *Rubus* sp.	AS05-27 – AS05-40
Loc. 4	Spain, Asturias, Somiedo, Puerto de Somiedo	42.99541° N 06.20290° W	1427 m	4 Dec. 2005	Shrubland with *Erica* spp., *Juniperus* sp., *Calluna vulgaris*, *Vaccinium* sp.	AS05-41 – AS05-53
Loc. 5	Spain, Asturias, Somiedo, Saliencia, Endriga	43.10909° N 06.15511° W	1300 m	5 Dec. 2005	Mixed forest with *Corylus avellana*, *Fraxinus excelsior*, *Genista occidentalis*	AS05-54 – AS05-63
Loc. 6	Spain, Asturias, Somiedo, Saliencia, Endriga	43.09000° N 06.15475° W	1120 m	5 Dec. 2005	Mixed forest with *Fagus sylvatica*, *Corylus avellana*	AS05-64 – AS05-69
Loc. 7	Spain, Asturias, Somiedo, Braña Campa d'Abaxu	43.07860° N 06.13067° W	1202 m	5 Dec. 2005	Livestock farm	AS05-70 – AS05-71
Loc. 8	Spain, Asturias, Somiedo, Saliencia lakes	43.05541° N 06.09935° W	1610 m	5 Dec. 2005	Subalpine shrubland	AS05-72 – AS05-78
Loc. 9	Spain, Asturias, Somiedo, Alto de la Farragona	43.06147° N 06.09975° W	1549 m	5 Dec. 2005	Mixed forest with *Sorbus aria*, *S. aucuparia*, *Ilex aquifolia*	AS05-79 – AS05-84
Loc. 10	Spain, Asturias, Somiedo, La Malva electric power station	43.11275° N 06.24660° W	700 m	5 Dec. 2005	Planted trees	AS05-85 – AS05-95
Loc. 11	Spain, Asturias, Somiedo, La Venta Castru, road to Pineda	43.12916° N 06.26738° W	534 m	6 Dec. 2005	Path in mixed forest with *Castanea sativa*	AS05-96 – AS05-108
Loc. 12	Spain, Asturias, Somiedo, Río Pigüeña	43.14482° N 06.33294° W	569 m	6 Dec. 2005	Riverside forest	AS05-109 – AS05-121

the more common species in ground litter and aerial litter, whereas more sampling effort is needed for bark.

In 60 of the 68 samples of substrate that were plated (Table 2), one or more species of protostelids fruited, that makes an 88 % of positive cultures for protostelids (PCP = number of collections positive for protostelids × 100/number of collections plated). Of these 68 plates, 30 were prepared using ground litter samples, 32 came from aerial litter samples, and six were from bark. The mean number of species occurring per plate was 2.41 (range 0–9) and the ratio between number of species recorded and plates was 0.31.

In five of the studied localities (Table 3) PCP was 100 %, and in all localities it was more than 72 %, except for one locality (Loc. 7, a livestock farm) where only one collection was plated, yielding negative results. The PCPs vary between the three microhabitats studied (Table 2): 93 % for ground litter samples, 81 % for aerial litter samples, and 100 % for bark. The latter is only an approximation due to the small number of samples, and it cannot be reliably compared with the other microhabitats, but marks a tendency.

Aerial litter (Table 2) constitutes the microhabitat with the highest species richness (16) and number of identifications (species recorded as present on a given sample of substrate) (75), followed by ground litter with 14 species and 72 identifications. It is remarkable that bark has very high species richness (ten), if we take the number of collections plated (six) and the number of identifications (17) into consideration.

The most commonly encountered species (Table 2) are *Protostelium mycophaga* (Pm) with 33 identifications, representing a 20 % of the total number of occurrences, *Schizoplasmodiopsis amoeboidea* (Sa) with 22 identifications (13 %), *S. pseudoendospora* (Sps) with 22 identifications (13 %), and *Soliformovum irregulare* (Si) with 17 identifications (10 %). All these species together with *Cavostelium apophysatum* and *Schizoplasmodiopsis cavostelioides* have been found in the three microhabitats. *Protostelium mycophaga* and *S. irregulare* seem to have preference for aerial microhabitat. *Endostelium zonatum*, *Nematostelium gracile*, and *Protostelium okumukumu* were recovered only from aerial litter samples. *Microglomus paxillus*, *Protostelium arachisporum*, and *Protosporangium fragile* were found exclusively on bark. *Nematostelium ovatum* and *Schizoplasmodiopsis micropunctata* were recovered exclusively from ground litter samples.

Localities can not be reliably compared because the number of samples is different in each case, but some preliminary data can be obtained. Localities with the highest species richness (Table 3) are Loc. 11 (with 12 species), Loc. 6 (11 species), Loc. 12 (11 species) and Loc. 1 (ten species). All of them are mixed broadleaf forests, except Loc. 12 that is a riverside forest. The highest number of identifications was found in Loc. 11 (31 identifications), Loc. 12 (27 identifications), Loc. 1 (19 identifications) and Loc. 10 (18 identifications), followed by Loc. 6 with 17 identifications. The highest number of species recorded from one sample was nine, for the sample AS05-96 (Loc. 11, *Rubus* sp., aerial litter).

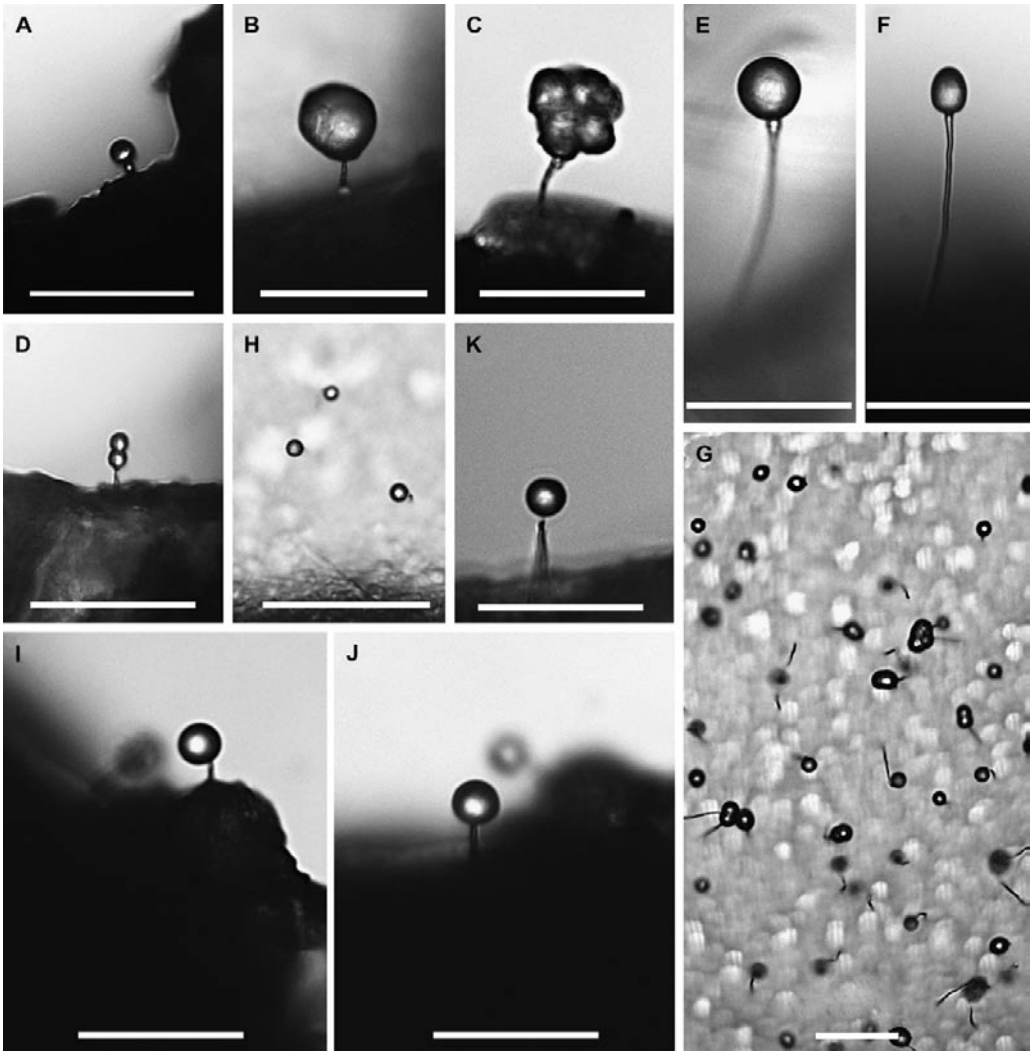

Fig 1 – Fruiting bodies of: (A) *Cavostelium apophysatum*; (B) *Echinosteliopsis oligospora* hydrated and (C) dried; (D) *Echinostelium bisporum*; (E) *Nematostelium gracile*; (F) *Nematostelium ovatum*; (G) *Protostelium mycophaga*; (H) *Protostelium nocturnum*; (I–J) *Schizoplasmodiopsis amoeboidea*; (K) *Schizoplasmodiopsis micropunctata*. Bars = 50 μm.

Taxonomy

A total of 21 species of protostelids were recorded. All of them are new records for southwestern Europe and seven are reported for the first time in Europe (noted with an asterisk).

Annotated species list

***Cavostelium apophysatum** L. S. Olive 1965
Loc. 1, ground litter of *Asteraceae*, AS05-12 (Fig 1A)
Loc. 3, aerial litter of *Lamiaceae*, AS05-39
Loc. 6, bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68
Loc. 9, ground litter of *Cytisus* sp., AS05-84; ground litter of *Tilia* sp., AS05-105

The apophysis, although usually wider than the base of the stalk, is sometimes narrow such that the stalk appears to be equally thick for its entire length. The spore is rough and, when observed in apical view, it appears nearly opaque.

Echinosteliopsis oligospora D. J. Reinh & L. S. Olive 1967
Loc. 1, ground litter of *Asteraceae*, AS05-12 (Fig 1B–C)
Loc. 2, aerial litter of *Cytisus* sp., AS05-20
Loc. 3, aerial litter of *Cytisus* sp., AS05-31; aerial litter of *Quercus ilex*, AS05-37

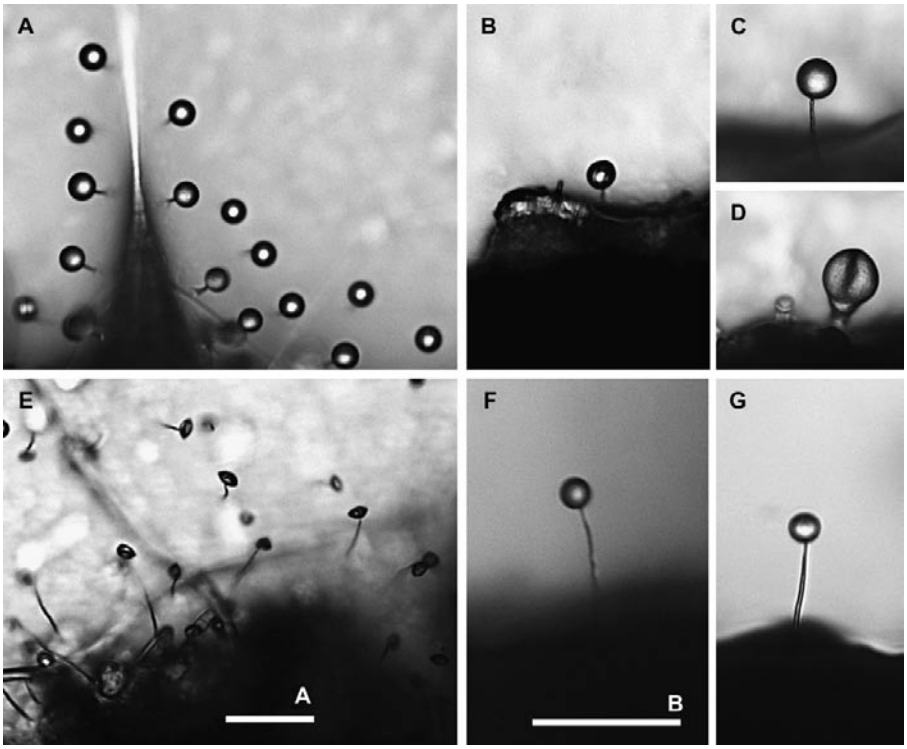

Fig 2 – Fruiting bodies of: (A–B) *Schizoplasmodiopsis pseudoendospora*; (C) *Schizoplasmodiopsis vulgare*; (D) *Schizoplasmodium cavostelioides*; (E) *Soliformovum irregulare* dried and (F) hydrated; (G) *Tychosporium acutostipes*. Bars = 50μm.

Loc. 11, ground litter of *Rubus* sp., AS05-97; ground litter of *Campanula* sp., AS05-101; ground litter of *Asteraceae*, AS05-103; aerial litter of *Tilia* sp., AS05-104

The number of spores is variable (4–8), and they are surrounded by a transparent, hygroscopic sheath. In conditions of high humidity the sheath appears as a spherical structure that contains the spores. In dryer conditions the sheath deflates and the sporangium becomes trefoil-shaped. In Europe this species has been reported previously from Germany (Tesmer *et al.* 2005).

Echinostelium bisporum (L. S. Olive & Stoian.) K. D. Whitney & L. S. Olive 1982 (Fig 1D)
Loc. 2, aerial litter of *Cytisus* sp., AS05-20
Loc. 10, aerial litter of *Poaceae*, AS05-87
Loc. 11, aerial litter of *Rubus* sp., AS05-96
Loc. 12, ground litter of *Rubus* sp., AS05-110

This mycetozoan was first described as a protostelid by Olive & Stoianovitch (1966) but it is now included in the myxomycetes (Spiegel & Feldman 1989; Whitney *et al.* 1982). It is usually studied under the same conditions as protostelids and usually grows intermixed with them. In Europe this species has been reported only from Germany (Tesmer *et al.* 2005).

*__Endostelium zonatum__ (L. S. Olive & Stoian.) W. E. Benn. & L. S. Olive 1984
Loc. 6, aerial litter of *Fagus sylvatica*, AS05-64

This species was found only once during our study but it presented the characteristic chain-like stalk and the pyriform spore that are typical of *E. zonatum* (Olive & Stoianovitch 1969).

*__Microglomus paxillus__ L. S. Olive & Stoian. 1977
Loc. 2, bark of *Crataegus monogyna*, AS05-26
Loc. 12, bark of *Alnus* sp., AS05-115

The 2–4 spores of this species can be observed through the sporangial sheath, that forms a round structure slightly flattened in the upper side.

Nematostelium cfr. **gracile** (L. S. Olive & Stoian.) L. S. Olive & Stoian. 1970 (Fig 1E)
Loc. 3, aerial litter of *Lamiaceae*, AS05-39

This species and *Ceratiomyxella tahitiensis* have identical fructifications and usually must be distinguished in culture. Unfortunately, all attempts to culture it failed, so its identity could not be confirmed. Spiegel *et al.* (2005) report that the vast majority of culture attempts are assigned to *N. gracile*, but all cultures from various parts of the world that have

Table 2 – Number of identifications per species in the three studied microhabitats

	Ca	Eo	Eb	Ez	Mp	Ng	No	Pfrag	Pa	Pm	Pn	Po	Ppyr	Sa	Sm	Sps	Sv	Sc	Se	Si	Ta	TI	R	NC	NP	PCP (%)
Ground litter	3	4	1	-	-	-	2	-	-	12	6	-	1	10	1	10	5	1	-	6	10	72	14	30	28	93
Aerial litter	2	4	3	1	-	1	-	-	-	20	5	1	3	8	-	8	1	3	1	10	4	75	16	32	26	81
Bark	1	-	-	-	2	-	-	1	1	1	-	-	-	4	-	4	-	1	1	1	-	17	10	6	6	100
TI	6	8	4	1	2	1	2	1	1	33	11	1	4	22	1	22	6	5	2	17	14	164	21	68	60	88

Ca, *Cavostelium apophysatum*; Eo, *Echinosteliopsis oligospora*; Eb, *Echinostelium bisporum*; Ez, *Endostelium zonatum*; Mp, *Microglomus paxillus*; Ng, *Nematostelium gracile*; No, *N. ovatum*; Pfrag, *Protosporangium fragile*; Pa, *Protostelium arachisporum*; Pm, *Protostelium mycophaga*; Pn, *P. nocturnum*; Po, *P. okumukumu*; Ppyr, *P. pyriforme*; Sa, *Schizoplasmodiopsis amoeboidea*; Sm, *Schizoplasmodiopsis micropunctata*; Sps, *S. pseudoendospora*; Sv, *S. vulgare*; Sc, *Schizoplasmodium cavostelioides*; Se, *Soliformovum expulsum*; Si, *S. irregulare*; Ta, *Tychosporium acutostipes*; TI, total number of identifications; R, species richness; NC, number of collections plated; NP, number of collections positive for protostelids; PCP, percentage of cultures positive for protostelids.

been established in the Spiegel laboratory in the last year have proven to have the amoeboflagellate state indicative of *C. tahitiensis* (Olive & Stoianovitch 1971). Further work is under way to clarify the taxonomy of protostelids with this sporocarp morphology.

In Europe, this species has been cited only from Germany (Tesmer *et al.* 2005).

Nematostelium ovatum (L. S. Olive & Stoian.) L. S. Olive & Stoian. 1970 (Fig 1F)

Loc. 6, ground litter of *Fagus sylvatica*, AS05-65

Loc. 10, ground litter of *Tilia* sp., AS05-94

This species has an ovoid or ellipsoid spore and a long, thick, robust stalk with a distinct apophysis. It has been recorded previously in Germany (Tesmer *et al.* 2005).

***Protosporangium fragile** L. S. Olive & Stoian. 1972

Loc. 2, bark of *Crataegus monogyna*, AS05-26

This species has a long, easily fragmented stalk that supports a four-spored sporocarp. It was found only once during our study.

Protostelium arachisporum L. S. Olive. 1962

Loc. 10, bark of *Pinus sylvestris*, AS05-95

The spores are very variable in shape, from almost spherical or ovate to elongate with one or more constrictions resembling the pod of a peanut. Tesmer *et al.* (2005) reported this species from Germany.

Protostelium mycophagum L. S. Olive & Stoian. 1960

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5; (Fig 1G) ground litter of *Pteridium aquilinum*, AS05-6; ground litter of *Asteraceae*, AS05-12; aerial litter of *Asteraceae*, AS05-11

Loc. 2, aerial litter of *Cytisus* sp., AS05-20; ground litter of thistle, AS05-23; ground litter of *Crataegus monogyna*, AS05-25

Loc. 3, ground litter of *Cytisus* sp., AS05-32; aerial litter of *Hedera helix*, AS05-35; aerial litter of *Lamiaceae*, AS05-39

Loc. 4, aerial litter of *Erica* sp., AS05-48; aerial litter of *Mentha* sp., AS05-52; ground litter of *Mentha* sp., AS05-53

Loc. 5, aerial litter of *Corylus avellana*, AS05-62

Loc. 6, aerial litter of *Fagus sylvatica*, AS05-64

Loc. 9, aerial litter of *Lamiaceae*, AS05-81; aerial litter of *Lamiaceae*, AS05-82; aerial litter of *Cytisus* sp., AS05-83

Loc. 10, aerial litter of *Poaceae*, AS05-87; aerial litter of *Aesculus hippocastanum*, AS05-88; bark of *Pinus sylvestris*, AS05-95

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; aerial litter of *Asteraceae*, AS05-102; ground litter of *Asteraceae*, AS05-103; aerial litter of *Tilia* sp., AS05-104

Loc. 12, aerial litter of *Rubus* sp., AS05-109; ground litter of *Rubus* sp., AS05-110; aerial litter *Lamiaceae*, AS05-111; aerial litter of *Alnus* sp., AS05-113; ground litter of *Equisetum* sp., AS-121

Very variable in size and deciduousness of spores. Some individuals seemed to have stalks that move easily in air

Table 3 – Occurrence of protostelid species in the 12 studied localities

	Ca	Eo	Eb	Ez	Mp	Ng	No	Pfrag	Pa	Pm	Pn	Po	Ppyr	Sa	Sm	Sps	Sv	Sc	Se	Si	Ta	TI	R	NC	NP	PCP (%)
Loc.1	1	1	-	-	-	-	-	-	-	4	1	-	-	2	-	3	3	2	-	1	1	19	10	4	4	100
Loc.2	-	1	1	-	1	-	-	1	-	3	1	-	-	3	-	-	-	-	-	2	1	14	9	6	5	83
Loc.3	1	2	-	-	-	1	-	-	-	3	2	-	1	-	-	1	-	-	-	2	1	14	9	7	6	86
Loc.4	-	-	-	-	-	-	-	-	-	3	-	-	-	4	-	2	-	-	-	1	1	11	5	7	5	72
Loc.5	-	-	-	-	-	-	-	-	-	1	-	-	2	1	-	-	-	-	-	1	-	5	4	2	2	100
Loc.6	2	-	-	1	-	-	1	-	-	1	-	-	-	2	-	3	1	2	1	1	2	17	11	6	5	83
Loc.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	1	0	0
Loc.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	1	100
Loc.9	1	-	-	-	-	-	-	-	-	3	-	-	-	2	-	-	-	-	-	-	1	7	4	6	5	83
Loc.10	-	-	1	-	-	-	1	-	1	3	1	-	-	5	-	5	-	-	-	-	1	18	8	11	10	91
Loc.11	1	4	1	-	-	-	-	-	-	5	5	1	1	2	-	4	-	-	1	3	3	31	12	7	7	100
Loc.12	-	-	1	-	1	-	-	-	-	7	1	-	-	1	1	4	2	1	-	5	3	27	11	10	10	100
TI	6	8	4	1	2	1	2	1	1	33	11	1	4	22	1	22	6	5	2	17	14	164	21	68	60	88
NL	5	4	4	1	2	1	2	1	1	10	6	1	3	9	1	7	3	3	2	9	9					

Ca, *Cavostelium apophysatum*; Eo, *Echinosteliopsis oligospora*; Eb, *Echinostelium bisporum*; Ez, *Endostelium zonatum*; Mp, *Microglomus paxillus*; Ng, *Nematostelium gracile*; No, *N. ovatum*; Pfrag, *Protosporangium fragile*; Pa, *Protostelium arachisporum*; Pm, *Protostelium mycophaga*; Pn, *P. nocturnum*; Po, *P. okumukumu*; Ppyr, *P. pyriforme*; Sa, *Schizoplasmodiopsis amoeboidea*; Sm, *Schizoplasmodiopsis micropunctata*; Sps, *S. pseudoendospora*; Sv, *S. vulgare*; Sc, *Schizoplasmodium cavostelioides*; Se, *Soliformovum expulsum*; Si, *S. irregulare*; Ta, *Tychosporium acutostipes*; TI, total number of identifications; R, species richness; NL, number of localities in were the species was found; NC, number of collections plated; NP, number of collections positive for protostelids; PCP, percentage of cultures positive for protostelids.

currents, whereas others had stiffer stalks. Sometimes two-spored fruiting bodies were observed. Frequently, sporocarps were found where spores germinated *in situ* and refruited, forming a chain. Usually this species appears in big dense patches covering large areas of the plate. In Europe, this species has been reported from Holland (Olive 1962, 1967), Sweden (Olive 1962, 1967), Greece (Olive 1967) and Germany (Tesmer *et al.* 2005).

Protostelium nocturnum Spiegel. 1984 (Fig 1H)

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5

Loc. 2, ground litter of thistle, AS05-23

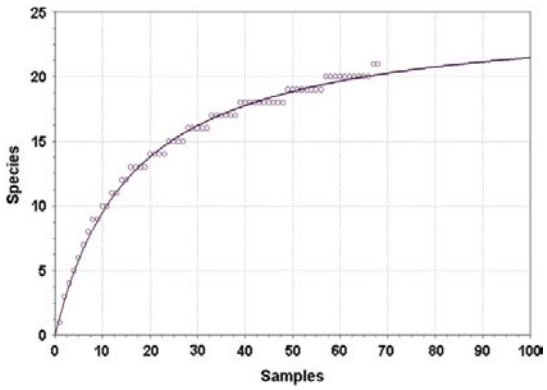

Fig 3 – BS analysis of the randomly permutated sequence of all samples studied versus cumulated species numbers (open circles). These values are the means of 100 runs. The solid line shows the results of regression analysis using a saturation function $y = Ax/(B+x)$, where A is the maximum number of species to be expected and B is the number of samples needed to reach half of the number of species to be expected.

Loc. 3, ground litter of *Cytisus* sp., AS05-32

Loc. 10, ground litter of *Tilia* sp., AS05-94

Loc. 12, aerial litter of *Lamiaceae*, AS05-111

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; aerial litter of *Asteraceae*, AS05-102; ground litter of *Asteraceae*, AS05-103; aerial litter of *Tilia* sp., AS05-104

Most of the patches of this species fruited most heavily after sunset until early morning. Spores are soon actively released with the disappearance of the stalk. In Europe, this species has been only reported from Germany (Tesmer *et al.* 2005).

*__Protostelium okumukumu__ Spiegel, Shadwick & Hemmes. 2006

Loc. 11, aerial litter of *Tilia* sp., AS05-104

This species has a bipartite stalk that supports a spherical spore. The spore is actively shot from the stalk with the disappearance of the spherical apophysis such that only the rigid basal portion of the stalk remains. In a patch of sporocarps, there is typically a dense stand of these stalk bases (Spiegel *et al.* 2006). This is the first confirmed observation of this recently described species (Spiegel *et al.* 2006) outside of Polynesia.

Protostelium pyriforme L. S. Olive & Stoian. 1969

Loc. 3, aerial litter of *Quercus ilex*, AS05-37

Loc. 5, aerial litter of *Corylus avellana*, AS05-62; ground litter of *Corylus avellana*, AS05-63

Loc. 11, aerial litter of *Rubus* sp., AS05-96

Sporocarps are similar in size to those of *P. mycophaga*. The spore is obpyriform or campanulate, often waving in air currents. In Europe, it has been previously reported only from Germany (Tesmer *et al.* 2005).

Schizoplasmodiopsis amoeboidea L. S. Olive & K. D. Whitney. 1982 (Fig 1I–J)

Loc. 1, ground litter of *Pteridium aquilinum*, AS05-6; ground litter of *Asteraceae*, AS05-12

Loc. 2, ground litter of *Cytisus* sp., AS05-21; ground litter of thistle, AS05-23; bark of *Crataegus monogyna*, AS05-26

Loc. 4, ground litter of *Calluna vulgaris*, AS05-42; bark of *Cytisus* sp., AS05-45; aerial litter of *Erica* sp., AS05-48; ground litter of *Lamiaceae*, AS05-53

Loc. 5, aerial litter of *Corylus avellana*, AS05-62

Loc. 6, aerial litter of *Fagus sylvatica*, AS05-64; bark of *Fagus sylvatica*, AS05-66

Loc. 9, aerial litter of *Cytisus* sp., AS05-83; ground litter of *Cytisus* sp., AS05-84

Loc. 10, aerial litter of *Erica arborea*, AS05-90; aerial litter of *Poaceae*, AS05-91; ground litter of *Poaceae*, AS05-92; ground litter of *Tilia* sp., AS05-94; bark of *Pinus sylvestris*, AS05-95

Loc. 11, aerial litter of *Rubus* sp., AS05-96; aerial litter of *Tilia* sp., AS05-104

Loc. 12, ground litter of *Alnus* sp., AS05-114

Sporocarps of this species have the same proportions as those of *S. pseudoendospora*. Most of them are bigger in size and grow in sparse patches. The stalk gets suddenly thinner towards the apex, forming a sharp point. Tesmer *et al.* (2005) reported this species for the first time in Europe.

***Schizoplasmodiopsis** cf. **micropunctata** L. S. Olive & Stoian. 1976 (Fig 1K)

Loc. 12, ground litter of *Lamiaceae*, AS05-112

The stalk of this species gets thinner in the apex, forming a hair-like structure at the point of attachment with the spore. The stalk in this material is more robust than usual (Spiegel *et al.* 2005).

Schizoplasmodiopsis pseudoendospora L. S. Olive, M. Martin. & Stoian. 1967 (Fig 2A–B)

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of *Asteraceae*, AS05-11

Loc. 3, aerial litter of *Cytisus* sp., AS05-31

Loc. 4, ground litter of *Calluna vulgaris*, AS05-42; bark of *Cytisus* sp., AS05-45

Loc. 6, ground litter of *Fagus sylvatica*, AS05-65; bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68

Loc. 10, ground litter of *Picea abies*, AS05-85; bark of *Picea abies*, AS05-86; ground litter of *Aesculus hippocastanum*, AS05-89; aerial litter of *Erica arborea*, AS05-90; ground litter of *Tilia* sp., AS05-94

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Rubus* sp., AS05-97; aerial litter of *Tilia* sp., AS05-104; ground litter of *Tilia* sp., AS05-105

Loc. 12, aerial litter of *Rubus* sp., AS05-109; bark of *Alnus* sp., AS05-115

This species tends to fruit in big dense patches, and is usually smaller than *S. amoeboidea*. It has been cited for Germany (Tesmer *et al.* 2005) and Ukraine (Glustchenko *et al.* 2002).

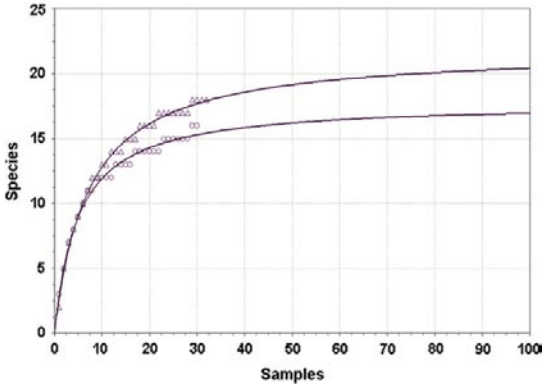

Fig 4 – BS analysis of the randomly permutated sequence of samples versus cumulated species numbers. Open circles represent the values for samples from the ground litter microhabitat and triangles represent the values for samples from the aerial litter microhabitat. These values are the means of 100 runs. The solid lines show the results of two regression analyses using a saturation function $y = Ax/(B+x)$, where A is the maximum number of species to be expected and B is the number of samples needed to reach half of the number of species to be expected.

Schizoplasmodiopsis vulgaris L. S. Olive & Stoian. 1976 (Fig 2C)

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; ground litter of *Asteraceae*, AS05-12

Loc. 6, ground litter of *Fagus sylvatica*, AS05-65

Loc. 12, ground litter of *Rubus* sp., AS05-110; ground litter of *Equisetum* sp., AS-121

The spores of this species are nearly spherical and the stalk length is variable. This species has been cited for England (Olive 1975b) and Germany (Tesmer *et al.* 2005).

Schizoplasmodium cavostelioides L. S. Olive & Stoian. 1966 (Fig 2D)

Loc. 1, ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of *Asteraceae*, AS05-11

Loc. 6, bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68

Loc. 12, aerial litter of *Rubus* sp., AS05-109

The spores attach to the stalk by a ring-shaped hilum that fits a distinct cup-shaped apophysis. In Europe, this species has been reported previously from Germany (Tesmer *et al.* 2005).

***Soliformovum expulsum** (L. S. Olive & Stoian.) Spiegel. 1994

Loc. 6, bark of *Fagus sylvatica*, AS05-66

Loc. 11, aerial litter of *Rubus* sp., AS05-96

The sporocarps are in the size range of *P. mycophaga*, but the stalk is bipartite with a broadly tapered basal section and a uniformly thin apical section. The stalk is usually

reflexed at the junction of the two sections. The spores are forcibly discharged with the disappearance of the stalk. The presence of ''fried egg''-shaped prespore cells helps to identify this species (Spiegel *et al.* 2005). It has been found only once during our study. Our material has an articulated stalk that bears a spherical spore that is typical of the species.

Soliformovum irregulare (L. S. Olive & Stoian.) Spiegel. 1994 (Fig 2E–F)

Loc. 1, aerial litter of *Asteraceae*, AS05-11

Loc. 2, aerial litter of *Cytisus* sp., AS05-20; bark of *Crataegus monogyna*, AS05-26

Loc. 3, aerial litter of *Cytisus* sp., AS05-31; aerial litter of *Lamiaceae*, AS05-39

Loc 4, aerial litter of *Mentha* sp., AS05-52

Loc. 5, ground litter of *Corylus avellana*, AS05-63

Loc. 6, aerial litter of *Erica* sp., AS05-68

Loc. 8, aerial litter of *Poaceae*, AS05-77

Loc. 11, aerial litter of *Rubus* sp., AS05-96; aerial litter of *Asteraceae*, AS05-102; aerial litter of *Tilia* sp., AS05-104

Loc. 12, ground litter of *Lamiaceae*, AS05-112; ground litter of *Alnus* sp., AS05-114; ground litter of *Equisetum* sp., AS-121

This is one of the tallest protostelids, and the stalks are usually very straight. Sometimes the hastate apophysis that is diagnostic of this species (Olive & Stoianovitch 1969; Spiegel *et al.* 1994) is not clearly obvious, and the stalk gets gradually thinner all the way to its apex. The deciduous spore can adhere to the side of the stalk after falling. When dried, it is ''American football''-shaped. In Europe, this species have been cited only from Germany (Tesmer *et al.* 2005).

Tychosporium acutostipes Spiegel, D. L. Moore & J. Feldman. 1995 (Fig 2G)

Loc. 1, ground litter of *Pteridium aquilinum*, AS05-6,

Loc. 2, ground litter of *Cytisus* sp., AS05-21

Loc. 3, aerial litter of *Lamiaceae*, AS05-39

Loc. 4, ground litter of *Lamiaceae*, AS05-53

Loc. 6, aerial litter of *Erica* sp., AS05-68

Loc. 9, ground litter of *Gentiana lutea*, AS05-80

Loc. 10, ground litter of *Picea abies*, AS05-85

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; ground litter of *Asteraceae*, AS05-103

Loc. 12, aerial litter of *Lamiaceae*, AS05-111; ground litter of *Lamiaceae*, AS05-112

Our specimens have a stiff stalk that gets gradually thinner towards its apex and is characteristic of the species (Spiegel *et al.* 1995). The spore can be somewhat turbinate. *Tychosporium acutostipes* has been recently cited for the first time in Europe (Tesmer *et al.* 2005).

Discussion

This study area has shown the highest species richness (21 species) recorded to date for Europe (Spiegel, unpubl.) or for a latitude this high (>40°). This number of species represents approximately 65 % of the described microscopic protostelid species of the world. Comparable species richness has been reported for the island of Hawaii (32 spp.) and Puerto Rico (25 spp.) in the tropics (Spiegel *et al.* 2006; Stephenson *et al.* 2004) and unpublished work of Shadwick & Spiegel has recorded 22 species in the Great Smoky Mountains National Park, USA, a mix of temperate forest habitats. Of these, only the last was of an area of comparable latitude (36°N) and scale.

Previous studies that have been carried out at comparable scale and effort in other parts of the world show, for instance, these values: 16 species from Hueston Woods State Park, Ohio, USA (Best & Spiegel 1984), 17 species were recovered from samples from Costa Rica (Stephenson & Moore 1998), 16 species from Northwest Arkansas (Moore & Spiegel 2000a), 15 from Germany (Tesmer *et al.* 2005), 13 from Caribbean National Forest, Puerto Rico (Moore & Spiegel 2000b; Stephenson *et al.* 1999), and 12 from northern India (Shadwick & Stephenson 2004). These results are evidence consistent with a hypothesis that Biosphere Reserves, such as Somiedo, are as important for maintaining the biodiversity of microorganisms as they are for the diversity of macroscopic organisms (SIAPA, 2004: http://tematico.princast.es/mediambi/siapa/web/espacios/espacios/pnt/somiedo/). This high richness in Somiedo could be a result of its proximity to the Mediterranean Basin, one of the world biodiversity hotspots (http://www.biodiversityhotspots.org/xp/Hotspots).

The highest species richness and number of identifications were found in aerial litter microhabitat, as reported in many other study areas (Moore & Spiegel 2000b; Moore & Stephenson 2003; Olive 1975a). It has been suggested that this tendency may be because some species of protostelids are unable to cope with antagonistic microorganisms in substrates on the ground (Olive 1975a); however, ground litter microhabitats are richest at very high latitudes (Spiegel & Stephenson 2000), and certain species are more commonly found in ground litter than other microhabitats (Moore & Spiegel 2000a). Another possible cause of this phenomenon is that the much more fluctuating moisture gradient of aerial litter could favour protostelids due to their short life cycles (Tesmer *et al.* 2005).

Protostelium mycophaga, *Schizoplasmodiopsis pseudoendospora*, and *Soliformovum irregulare* are very frequently encountered species in the Somiedo Biosphere Reserve, as well as in other temperate areas (Best & Spiegel 1984; Moore & Spiegel 2000a; Tesmer *et al.* 2005), but *S. amoeboidea* appears more frequently here than has been reported in other parts of the world. Perhaps it was caused by the long drought period that had taken place before sampling. *Protostelium mycophaga* is possibly the most common protostelid worldwide, and appeared in all the substrate types examined and in ten of the 12 sampling localities as well.

These promising results, though still preliminary, can constitute a basis for further research and suggest that the north of Spain, a transitional area between boreal and Mediterranean vegetation, can be a very interesting place for further work. Spain, one of the European areas with highest biodiversity of other organisms, also appears to have high protostelid richness. It is an excellent location to study the biology of this group in more detail and its wide variety of habitats and

climatic regions can help to increase the ecological information on these organisms.

Acknowledgements

We wish to thank Diana Wrigley de Basanta for her helpful advice and language corrections and Juan Carlos Hernández developing a program for the data analysis. This work has been supported by the Research Project (CGL2005-00320/BOS) of the Ministry of Education and Science of Spain and US National Science Foundation grants DEB 0316284 and DEB 0329102 .

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Capítulo 2: Optimización del Método de Cultivo

Tras comprobar la presencia de amebas protosteloides en la Península Ibérica, y ver sobre qué tipo de sustratos podían aparecer, fue posible realizar un muestreo más amplio en una zona con clima mediterráneo del centro peninsular. Al cultivar las primeras muestras procedentes de esta nueva área se encontraron prácticamente las mismas especies que en los cultivos de muestras del Parque Natural de Somiedo (Capítulo 1). Sin embargo, al comparar las abundancias de las especies en las dos zonas se observaron cambios. Los trabajos anteriormente publicados sobre protostélidos mostraban diferencias importantes entre microhábitats a escala local y al mismo tiempo, al comparar estudios de zonas muy alejadas entre sí, ciertas tendencias que podrían responder a patrones latitudinales o a preferencias individuales de las especies por ciertos tipos de clima (Spiegel et al, 2007; Ndiritu et al, 2009a). Nos planteamos entonces las siguientes preguntas: ¿Podrían entonces las diferencias en el clima explicar la variación en las abundancias de protostélidos entre distintas zonas de la Península Ibérica? ¿Cómo afectaría el clima a las comunidades de protostélidos presentes en cada uno de los microhábitat estudiados? ¿Cuáles son las preferencias ecológicas en cuanto a microhábitat y clima de cada especie en la Península Ibérica?

Para estudiar estos aspectos de la ecología de las amebas protosteloides se hacía necesario utilizar un método de muestreo y de cultivo que permitieran obtener suficiente información sobre las especies, adecuando el esfuerzo a los objetivos de nuestro estudio, y que permitiera que los datos obtenidos en las distintas localidades fueran comparables. Por ello, se optó por intentar recoger el mismo número de muestras en cada microhábitat para cada localidad estudiada siempre que fuera posible, y por realizar con estas primeras muestras procesadas una optimización del esfuerzo de cultivo necesario para alcanzar los objetivos planteados. Finalmente, se realizaron los primeros análisis, para comprobar que las metodologías escogidas permitían obtener conclusiones sobre la ecología de estos organismos.

Los resultados obtenidos se publicaron en un nuevo artículo:

Aguilar, M., Spiegel, F. W. & Lado, C. (2011). Microhabitat and climatic preferences of Protosteloid Amoebae in a region with a Mediterranean climate. Microbial Ecology 62(2):361-373.

Resumen: El papel del microhábitat y la variación del clima en la estructuración de las comunidades de amebas protosteloides ha sido investigado por primera vez en la

región mediterránea, un punto caliente de biodiversidad de plantas y animales y la mayor de las cinco áreas del mundo con clima mediterráneo. Se obtuvieron datos de abundancia usando sustratos naturales recolectados en 13 localidades del centro de España, y se registraron un total de 1504 colonias y 18 especies. Para esta nueva área, se ha realizado una optimización del esfuerzo de cultivo basada en análisis de rarefacción, haciendo así posible adaptar el protocolo a los objetivos en investigaciones futuras. Si el objetivo es encontrar la mayor parte de las especies raras, se recomienda usar dos placas por muestra con cuatro líneas de sustrato cada una. Si las especies raras no son esenciales, como ocurre en algunos estudios ecológicos, sólo una placa por muestra con cuatro líneas de sustrato es suficiente. Los análisis de correspondencia canónica y los modelos lineares generalizados realizados mostraron que el tipo de microhábitat es el factor más importante para diferenciar los nichos de las especies estudiadas, pero las variables climáticas, especialmente la temperatura mínima del mes más frío, tienen efectos secundarios pero también importantes. Las especies que viven sobre corteza tienden a ser más abundantes en las localidades con mayor rango de temperaturas y menor precipitación annual. La hojarasca aérea es el microhábitat con la mayor riqueza de especies, la mayor abundancia y la menor heterogeneidad. Las especies típicas de este microhábitat son más abundantes cuando hay mayor precipitación, menor temperatura del mes más cálido, y menor temperatura del mes más frio.

Microb Ecol
DOI 10.1007/s00248-011-9843-6

FUNGAL MICROBIOLOGY

Microhabitat and Climatic Preferences of Protosteloid Amoebae in a Region with a Mediterranean Climate

María Aguilar · Frederick W. Spiegel · Carlos Lado

Received: 16 November 2010 / Accepted: 2 March 2011

Abstract The role of microhabitat and climate variation in structuring protosteloid amoebae communities has been investigated for the first time in the Mediterranean Basin, a biodiversity hotspot for plants and animals and the largest of the world's five areas with a Mediterranean climate. Abundance data were obtained from natural substrates collected in 13 localities from central Spain, and a total of 1,504 colonies and 18 species were recorded. For this new area, it has been carried out an optimization of the culturing effort based on rarefaction analyses, thus making possible to adapt the protocol to the objectives in future research. Canonical correspondence analysis and generalized linear models showed that microhabitat type was the most important factor for differentiating the niches of the species studied, but climatic variables, especially minimum temperature of the coldest month, precipitation seasonality, and temperature range, had secondary but also important effects. Bark inhabitants tend to be more abundant in localities with high temperature range and low annual precipitation. Aerial litter was the microhabitat with the highest species richness, abundance, and evenness. Species typical of this microhabitat are more abundant when there is high precipitation, low temperature of the warmest month, and low minimum temperature of the coldest month.

M. Aguilar (✉) · C. Lado
Departamento de Micología, Real Jardín Botánico, CSIC,
Plaza de Murillo 2,
28014 Madrid, Spain
e-mail: aguilar@rjb.csic.es

F. W. Spiegel
Department of Biological Sciences, University of Arkansas,
Fayetteville, AR 72701, USA

Published online: 22 March 2011

Introduction

Protosteloid amoebae, formerly called protostelids, are a diverse group of slime molds in the eukaryotic supergroup Amoebozoa [1, 31]. They produce simple, stalked fruiting bodies, known as sporocarps [23, 31, 33, 34]. The sporocarps always consist of a single acellular stalk and one to a few spores, but there is also a trophic stage that varies from uninucleate amoeboid and/or amoeboflagellate cells to multinucleate reticulate plasmodia [23, 31, 34]. All known protosteloid species are heterotrophic microorganisms and act as predators on terrestrial decomposers such as bacteria, yeasts, and spores of filamentous fungi [36]. Despite their morphological similarities and common lifestyle, recent molecular data suggest that they may have polyphyletic origins within the Amoebozoa [10, 31]. They can occur on many different microhabitats, such as dead aerial plant parts, bark, leaf litter, and soil [23, 36].

The ecology of this group has not been studied until recently [34], with most works focused on comparisons of species assemblages from different microhabitats at a local scale. A microhabitat is a small, localized habitat within a larger ecosystem, having conditions that sustain a limited range of organisms that form a distinct community. At different latitudes, species appear in samples from different microhabitats, and their relative abundance changes [21]. Though it has been pointed out that elevation and latitude could cause changes in species composition in a given microhabitat [21], the underlying influences of climatic factors have not been disentangled. Several studies have been made throughout the world in temperate areas [2, 3, 5, 15, 17, 18, 29, 30, 39], tropical regions [16, 19, 21, 24, 37], polar regions [20, 35], and aquatic environments [14, 40], but studies of protosteloid amoebae communities at a large scale

have not been made, due in part to the lack of comparable datasets.

In spite of all efforts, there are still many gaps in our knowledge of the distribution of protosteloid amoebae. No studies have taken place in a region with a Mediterranean climate, characterized by hot dry summers that contrast with cyclonic rains in winter [41]. There are five areas in the world with this kind of climate, all of them biodiversity hotspots for plants and animals [4] and located in the Mediterranean Basin, California (USA), parts of central Chile, the Cape region of South Africa, and areas in the south and southwest of Australia. For this study, we have selected the central area of Spain, in the Mediterranean Basin, to check if this area also harbors a high diversity of these organisms, and to provide an analysis of the diversity and ecology of protosteloid amoebae in this kind of climate. Spain has previously proved to be an excellent location for other groups of slime molds, such as dictyostelids [26] and myxomycetes [12, 13].

The objective of this paper is to report the differences in species composition and relative abundance of protosteloid amoebae between microhabitats, especially with respect to evaluating the influence of different climatic factors on these parameters. As these organisms have never been studied in localities with a Mediterranean climate and previous information about their ecology is limited, the sampling method has been emphasised, not only to test and find the optimum effort needed for the particularities of these areas but also to provide a more quantitative, statistical method that will allow comparison between different ecosystems in further studies. This optimization can be used in the future for designing new experiments in similar areas, adapting the effort to the objectives of the research.

Material and Methods

Sampling and Culturing

This study is based on material collected during two sampling efforts in 2006 and 2007 in two areas of central Spain (Fig. 1). Samples were collected in a total of 13 localities (Table 1), all georeferenced with a Garmin GPS 16, datum WGS 84, located in well-conserved areas between 40–41° N and 2–5° W in a range of altitude between 500 and 900 m and with different vegetation types. The first sampling (localities 1–7) took place in a region called "Alcarria." It extends principally over the province of Guadalajara but also enters Cuenca and Madrid. The second sampling (localities 8–13) comprised different locations in the west of the province of Madrid and the provinces of Toledo and Avila, in a natural region called

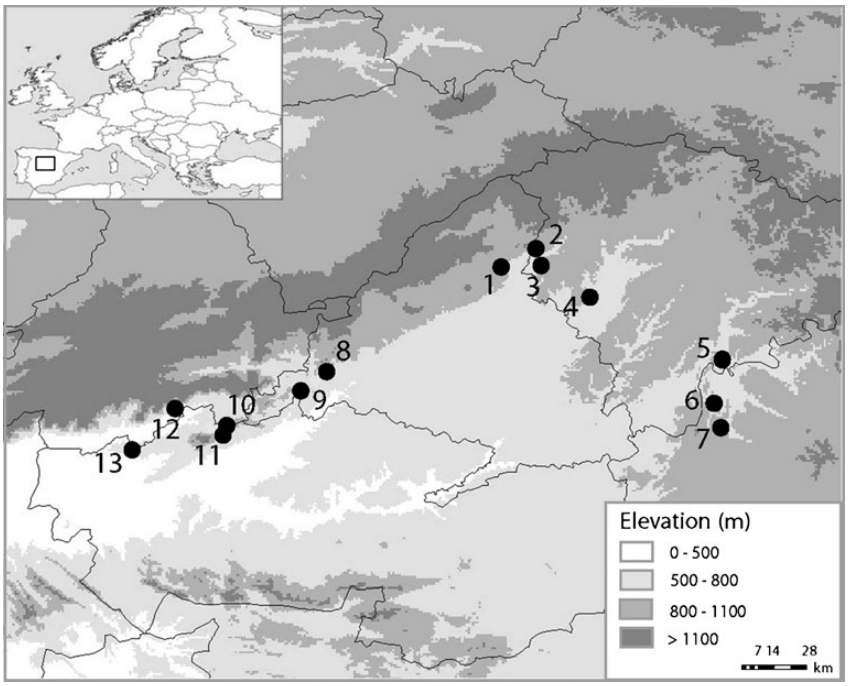

Figure 1 Studied localities. *Black circles* show the location of the 13 localities sampled (see Table 1)

Table 1 Sampled localities and their characteristics

	Coordinates	Elevation (m)	Date	Description	Sample codes
Loc. 1	40°48′45″ N, 03°35′16″ W	815±5	26 October 2006	Mediterranean shrubland, with *Quercus* and Labiatae	M06-29–M06-38
Loc. 2	40°53′09″ N, 03°27′19″ W	868±3	26 October 2006	Mediterranean shrubland, with *Cistus* spp.	M06-39–M06-44
Loc. 3	40°49′32″ N, 03°26′07″ W	880±7	26 October 2006	Mediterranean shrubland, with Labiatae	GU06-01–GU06-06
Loc. 4	40°41′56″ N, 03°14′39″ W	805±4	26 October 2006	Mediterranean shrubland, with *Quercus* sp.	GU06-07–GU06-10
Loc. 5	40°27′36″ N, 02°43′54″ W	765±4	26 October 2006	Mediterranean shrubland, with *Quercus* sp. and Labiatae	GU06-11–GU06-16
Loc. 6	40°17′24″ N, 02°45′47″ W	670±5	26 October 2006	Grassland in a hill, with Gramineae and Compositae	CU06-01–CU06-04
Loc. 7	40°12′06″ N, 02′44′22″ W	800±4	26 October 2006	Mediterranean shrubland, with Labiatae	CU06-05–CU06-08
Loc. 8	40°25′03″ N, 04′15′50″ W	787±4	19 February 2007	Mediterranean forest, with *Quercus ilex*	M07-01–M07-10
Loc. 9	40°20′25″ N, 04′21′42″ W	770±6	19 February 2007	Mediterranean forest, with *Pinus* sp.	M07-11–M07-20
Loc. 10	40°12′38″ N, 04′38′40″ W	640±9	19 February 2007	Mediterranean forest, with *Quercus ilex*	AV07-01–AV07-10
Loc. 11	40°10′14″ N, 04′39′40″ W	710±4	19 February 2007	Mediterranean forest, with *Quercus ilex*	TO07-01–TO07-10
Loc. 12	40°16′14″ N, 04′50′42″ W	680±13	19 February 2007	Mediterranean forest, with *Quercus pyrenaica* and *Pinus* sp.	AV07-11–AV07-20
Loc. 13	40°06′49″ N, 05′00′43″ W	530±6	19 February 2007	Mediterranean forest, with *Quercus ilex*	TO07-11–TO07-20

"La Vera." The climate in the two selected areas is Mediterranean continental, with long, dry, and warm summers and long cold winters. Springs and autumns are mild, humid, and short. The typical vegetation of these areas mainly consists of Mediterranean forests, most of them dominated by *Quercus ilex* or *Quercus faginea*. Due to historical agricultural activities, many of the original forests have disappeared, giving rise to ecosystems in different successional stages in which shrublands predominate. These shrublands are partially determined by the soil type, being Labiatae (*Rosmarinus*, *Thymus*, *Lavandula*, *Salvia*…) the dominant vegetational components in limestones and Cistaceae (*Cistus* spp.) and Leguminosae (*Retama*) in siliceous substrates.

A total of 100 samples (44 of ground litter, 44 of aerial litter, and 12 of bark) were collected. At each site, we intended to collect ten samples from three different microhabitats and different plant species. The objective was to obtain four samples of ground litter (the layer of twigs, leaves, and other plant debris extending over the soil surface), four samples of aerial litter (assemblage of dead but still attached parts of standing plants), and two samples of bark of living plants per locality. However, this was not always possible due to the absence of appropriate plant tissues. Collections of samples were placed in separate paper bags and air-dried in the laboratory of Real Jardín Botánico. These samples were stored there with the codes shown in Table 1.

Primary isolation plates were prepared between October 2006 and June 2007, using a modification of the technique described in [23] (see also [15] and [36]). The material was cut into small (ca. 1.5–2 cm) pieces with sterile scissors. Thirty-two pieces from each sample were plated out in eight lines of four pieces forming a circle on a 9-cm Petri dish (Fig. 2) with

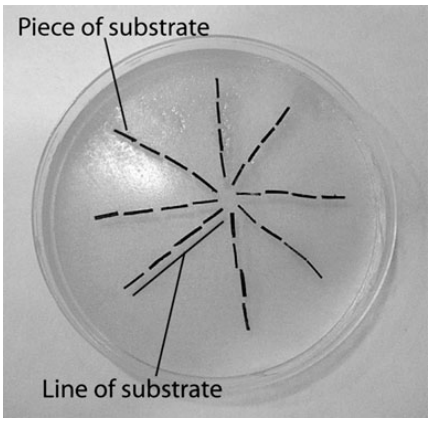

Figure 2 A primary isolation plate with thirty-two pieces of substrate, that were plated out in eight lines of four pieces forming a circle on a 9-cm Petri dish

a weakly nutrient medium (wMY—0.002 g malt extract, 0.002 g yeast extract, 0.75 g K_2HPO_4, 15 g agar/L of distilled water). The material was moistened by pipetting on a few drops sterile water per line. Three plates per sample were prepared, yielding a total of 300 plates (2,400 lines and 9,600 pieces of substrate). The plates were incubated at 21°C and were surveyed for protosteloid amoebae in the second week of culture.

Species were identified on the basis of fruiting body morphology under the light microscope using both unpublished [36] and original descriptions. Nomenclature used herein follows [23] and [11]. Colonies of protosteloid amoebae were counted in each line of substrate from each plate. A colony is defined as an individual fruiting body or a patch of fruiting bodies that is separated from the nearest fruiting body of the same species by at least one field of view under a ×10 objective on a compound microscope (i.e., approximately 2.0 mm) [15]. Colony size was not taken into account for abundance measures. Photomicrographs were taken with a Nikon Eclipse 80i compound microscope using bright field optics and a Nikon Digital Sight DS-5M digital camera.

Data Analysis

Species richness was calculated after considering only one randomly selected plate per sample, two randomly selected plates per sample, and finally all the plates cultured. This process was repeated 100 times with different random ordinations of the plates using the program R 2.6.2 [25]. The plot of the species richness vs. the number of plates per sample was subjected to a non-linear regression analysis with the program CurveExpert 1.3 [8], using as a saturation formula the Michaelis–Menten equation:

$$f(x) \sim y = Ax/(B+x)$$

In each case, the parameters A and B from the Michaelis–Menten formula, the standard error, and the coefficient of correlation were estimated. For a better evaluation of the results, the conditions necessary for obtaining an 80% and a 90% of the estimated maximum number of species were also calculated. Similarly, the cumulative species richness was also measured using 100 permutations with different numbers of lines of substrate per plate and per sample, using CurveExpert 1.3 to calculate the parameters A and B.

To evaluate the extent to which the survey was exhaustive and estimate the actual number of species, two methods were used—rarefaction and a nonparametric estimator. Both methods were used for studying all the samples together and samples from the three different microhabitats separately. For the first method [27, 28], the sequence of samples was randomly permutated 100 times, and the cumulative number of species was calculated for each permutation using R 2.6.2. The plot of the mean cumulative number of species vs. the number of samples was subjected to a regression analysis with the program CurveExpert 1.3, using the Michaelis–Menten equation. The number of species was also estimated using the abundance-based coverage estimator (ACE) [6, 7], with the program Spade [32] and a cutoff point=10.

Significance of the differences in abundance between ground litter and aerial litter was tested with a chi-square test for each species, using as expected frequency the average number of colonies between the two microhabitats. When the obtained p value was 0.5 or less in the chi-square test, the species were considered as having equal preferences for aerial litter and ground litter.

On the basis of their relative abundances, the species have been classified in the abundance classes described in [21]: abundant >10% of total colonies, common >5%, occasional >1%, and rare <1%. Abundance classes' boundaries were kept to be consistent with [21] and facilitate future work. Though they are informal, they provide a good tool for a quick and easy comparison of relative abundance of species between studies. A canonical correspondence analysis (CCA) was performed using abundant, common, and occasional species as dependent variables and annual mean temperature, annual precipitation, precipitation of the wettest month, precipitation of the

Table 2 Values of the climatic variables in each locality obtained from EDIT geoplatform

	T	*P*	PW	PD	PS	MTW	mTC	TR
Loc. 1	12.1	465	57	13	32	28.8	−0.1	28.9
Loc. 2	12.8	431	54	12	33	29.4	0.6	28.8
Loc. 3	12	463	56	14	31	28.8	−0.1	28.8
Loc. 4	12.5	435	54	12	33	29.3	0.3	29
Loc. 5	12.8	429	51	14	30	30.2	−0.1	30.1
Loc. 6	12.7	445	52	14	30	30.5	−0.1	30.6
Loc. 7	12.8	446	52	13	31	30.7	−0.1	30.8
Loc. 8	13.3	397	47	11	33	30.5	0.7	29.8
Loc. 9	12.4	411	52	12	34	29.8	−0.1	29.9
Loc. 10	13.9	373	46	9	35	31.7	1.1	30.6
Loc. 11	13.2	391	49	10	36	31.1	0.4	30.7
Loc. 12	13.8	371	46	9	36	31.5	11	30.4
Loc. 13	14.4	375	44	7	37	32.3	1.5	30.8

T annual mean temperature in degree Celsius, *P* annual precipitation in millimeters, *PW* precipitation of the wettest month in millimeters, *PD* precipitation of the driest month in millimeters, *PS* precipitation seasonality (coefficient of variation), *MTW* maximum temperature of the warmest month in degree Celsius, *mTC* minimum temperatures of the coldest month in degree Celsius, *TR* temperature range in degree Celsius

driest month, precipitation seasonality, maximum temperature of the warmest month, minimum temperature of the coldest month, temperature range, and microhabitat type as independent variables (Table 2), with R 2.6.2 and the vegan package [22]. Environmental data were obtained as raster layers from EDIT Geoplatform [9], and values for each sampling point were extracted using ArcGis from ESRI. Species were scaled proportional to eigenvalues, sites were unscaled (weighted dispersion equal on all dimensions), and permutation tests were carried out. For a better interpretation of the results, the correlation between all pairs of climatic variables was studied using regression analyses in R 2.6.2.

For each species, the probability distribution with the best fit was selected using various nonparametric statistics (maximum likelihood fitting, Kolmogorov–Smirnov test, chi-square test), and significance of the former climatic factors together with microhabitat type was tested using generalized linear models (GLM) in R 2.6.2. Only abundant, common, and occasional species were analyzed. Rare species were not considered in these analyses because there is not enough information about them to obtain reliable results.

Results

Optimization

The results of the optimization of the culture method are presented in Fig. 3 and Table 3.

The effect of culturing a different number of lines of substrate per sample has been studied using two different methods. The first one (Fig. 3b) studies the effect of always using three plates per sample, varying the number of lines that are cultured in each plate. Thus, this method takes into account in which plate the different substrate lines were initially cultured. The second method (Fig. 3c) varies the number of lines per sample selecting them randomly. Comparing the values in the table for obtaining an 80% of the species shown in Table 3, the number of lines per sample necessary (3.7) is less than three times the number of lines per plate (1.56 x 3 = 4.68). The same thing happens when observing the values in the 90% column, being the number of lines per sample (8.33) again less that three times the number of lines per plate (10.53). This effect shows that there are random differences between culture conditions that could cause that, using the same quantity of substrate, potentially more species can be obtained if the sample is divided and cultured in different plates.

The number of plates and the number of lines per plate have to be selected depending on the objectives of the study. If the main objective is finding as many species as possible, or isolating a specific rare organism, the culturing methodology should be adjusted for obtaining the maximum number of species with a reasonable effort. In this case, it is recommended to use two plates per sample to take advantage of random effects between plates. The use of three plates per sample produced an increment only of 2% (one species) with respect to the use of two plates per sample. For recovering at

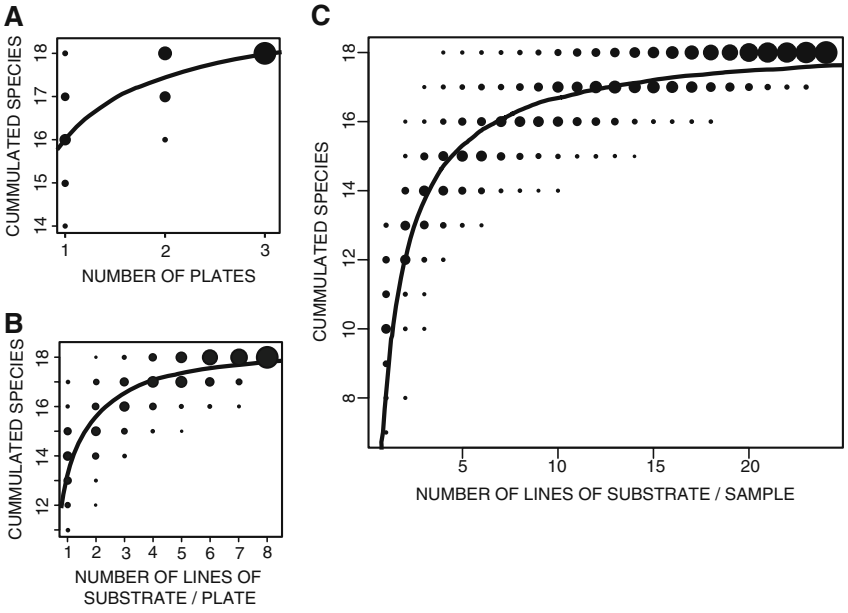

Figure 3 Optimization analyses. **a** Cumulative species vs. number of plates per sample. **b** Cumulative species vs. number of lines of substrate per plate, using three plates per sample. **c** Cumulative species vs. number of lines per sample. These values are the means of 100 runs. The *solid line* shows the results of regression analysis using a saturation function $y = Ax/(B + x)$, where A is the maximum number of species to be expected and B is the number of elements needed to reach half the number of species to be expected

Table 3 Results of the rarefaction analyses of the number of plates and the number of lines of substrate

	A	*B*	s.e.	c.c.	80%	90%
No of plates/sample	19.07	0.18	0.71	0.87	0.73	1.65
No of lines of substrate/plate	18.67	0.39	0.86	0.86	1.56	3.51
No of lines of substrate/sample	18.32	0.92	0.91	0.88	3.7	8.33

The cumulative species richness was measured using 100 permutations with different numbers of plates per sample, lines of substrate per plate, and lines of substrate per sample. Results were subjected to regression analyses using the Michaelis–Menten equation $f(x) \sim y = Ax/(B+x)$ as saturation formula

A, *B* parameters from Michaelis–Menten formula, *s.e.* standard error, *c.c.* correlation coefficient, *80%* number of plates or lines of substrate necessary for obtaining an 80% of the species, *90%* number of plates or lines of substrate necessary for obtaining a 90% of the species

least a 90% of the species, it is necessary to plate four lines of substrate per plate (eight in total).

If the goal of the study is characterizing the ecological preferences of a species or a group of species, it is necessary to obtain a sufficient number of occurrences of the organisms of interest. In this case, rare species are not good targets if the number of samples is limited because they are strongly affected by random errors. Using only one plate per sample made possible to recover an 85% of the species, that is, all of them except two of the rare species. Similarly, the results of changing the number of lines of substrate showed that 80% of the species are obtained using only four lines of substrate per sample. In conclusion, for an ecological study of the abundant, common, and occasional species, it is sufficient to use one plate per sample and four lines of substrate.

Ecology

Protosteloid amoebae fruited in 95 of the 100 samples collected. The percentage of cultures positive for protosteloid amoebae (PCP=number of primary isolation plates (PIP) positive for protosteloid amoebae×100/total number of PIP) was 84%. After observing three plates per sample, a total of 1,504 colonies were found (Table 4), from which 18 species were identified. The mean number of species occurring per sample was 4.24 (range 0–13).

All 18 observed species (Figs. 4 and 5) were recovered from the aerial litter microhabitat while only 15 of the species were found in ground litter samples and 11 in samples from bark. The number of colonies was also higher in aerial litter (904 [20.5/sample]) than in ground litter (551 [12.5/sample]) and bark (49 [4.1/sample]).

The number of species estimated with rarefaction and ACE are very close to the number of species recorded from the samples. Similar results were obtained using both methods, so it can be reliably concluded that we have recovered more than 90% of the total species that would have been found with much more effort employing the same methodology (Table 5; Fig. 6) The survey was exhaustive, especially for aerial litter and ground litter (more than 85–90% of the species). This is not the case for bark for which only a 70% of the estimated number of species was found. The evenness of communities can be compared by examining the steepness of the rarefaction curves (Fig. 6b). The curve is steeped in aerial litter than in ground litter and bark, indicating a more even distribution of species among samples in aerial litter.

The most commonly encountered and abundant species in this study (Table 4) were *Protostelium mycophaga* (34% of the total colonies), *Schizoplasmodiopsis pseudoendospora* (19%), *Tychosporium acutostipes* (11%), and *Schizoplasmodiopsis amoeboidea* (10%). *Cavostelium apophysatum* (9%), and *Nematostelium gracile* (6%) were common species, while *Nematostelium ovatum* (1%), *Protosporangium articulatum* (2%), *Protostelium nocturnum* (2%), *Schizoplasmodiopsis vulgare* (2%), and *Soliformovum irregulare* (1%) were occasional species. The remaining seven species were rare.

Two rare species, *Echinosteliopsis oligospora* and *Protostelium okumukumu*, were recovered only from aerial litter samples. The rare protosteloid myxomycete, *Echinostelium bisporum*, was found on both aerial litter and bark but not on ground litter. *N. ovatum*, *P. nocturnum*, *Protostelium pyriformis*, *Schizoplasmodium cavostelioides*, and *Soliformovum irregularis* were recovered from both aerial and ground litter samples, but not from bark. The remaining species were found in all three of the microhabitats that were studied.

The abundance of each species was significantly different in aerial and in ground litter microhabitats in most cases; only *C. apophysatum*, *S. amoeboidea*, and *Schizoplasmodium cavostelioides* had a $p<0.5$ in the chi-square test (Table 4) and showed no preference for aerial litter or ground litter. *N. ovatum* and *S. pseudoendospora* were significantly more abundant in the ground litter microhabitat, but the remaining species were significantly more common in aerial litter. Rarefaction analysis showed that a high percentage of the species predicted in bark were not found. As this incomplete sampling may also affect abundance data, chi-square tests including bark were not performed due to the small number of samples collected from this microhabitat.

Table 4 Number of colonies per species and microhabitat, absolute, and relative abundance

	A		G		B		Total	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
*Cavostelium apophysatum** (C)	65	1.48	67	1.52	8	0.67	140	1.4
Echinosteliopsis oligospora (R)	2	0.05	0	0	0	0	2	0.02
Echinostelium bisporum (R)	3	0.07	0	0	1	0.08	4	0.04
Endostelium zonatum (R)	8	0.18	2	0.05	2	0.17	12	0.12
Nematostelium gracile (C)	43	0.98	38	0.86	4	0.33	85	0.85
Nematostelium ovatum (O)	8	0.18	11	0.25	0	0	19	0.19
Protosporangium articulatum (O)	31	0.7	4	0.09	2	0.17	37	0.37
Protostelium arachisporum (R)	2	0.05	1	0.02	1	0.08	4	0.04
Protostelium mycophaga (A)	422	9.59	83	1.89	2	0.17	507	7.07
Protostelium nocturnum (O)	21	0.48	4	0.09	0	0	25	0.25
Protostelium okumukumu (R)	1	0.02	0	0	0	0	1	0.01
Protostelium pyriforme (R)	10	0.23	1	0.02	0	0	11	0.11
*Schizoplasmodiopsis amoeboidea** (A)	73	1.66	75	1.7	6	0.5	154	1.54
Schizoplasmodiopsis pseudoendospora (A)	78	1.77	182	4.14	21	1.75	281	2.81
Schizoplasmodiopsis vulgare (O)	22	0.5	9	0.2	1	0.08	32	0.32
*Schizoplasmodium cavostelioides*** (R)	1	0.02	1	0.02	0	0	2	0.02
Soliformovum irregulare (O)	18	0.41	4	0.09	0	0	22	0.22
Tychosporium acutostipes (A)	96	2.18	69	1.57	1	0.08	166	1.66
Total	904	20.55	551	12.52	49	4.08	1504	15.04

A aerial litter, *G* ground litter, *B* bark, (*A*) abundant, (*C*) common, (*O*) occasional, (*R*) rare

*$p<0.05$ (no significant differences between A and G; chi-square test); **$p<0.01$ (no significant differences between A and G; chi-square test)

The results of the correlation analysis between the climatic variables are shown in Fig. 7a. The variables annual mean temperature, precipitation of the wettest month, and maximum temperature of the wettest month are very highly correlated ($r^2>0.9$) so their individual effects on the species in the studied area cannot be easily distinguished.

The CCA (Fig. 7b) had a total inertia of 1.400, a constrained inertia of 0.223 (proportion 15.92%), and an unconstrained inertia of 1.177 (84.07%). Permutation tests were carried out; the test for the axes was significant ($p=0.005$), and the test for the independent variables showed that aerial litter microhabitat ($p=0.010$), annual mean temperature ($p=0.005$), maximum temperature of the warmest month ($p=0.030$), and minimum temperature of the coldest month ($p=0.015$) had significant effects. The variables that were more important for differentiating the niches of the studied species were the microhabitats, but it is interesting to observe that the climatic variables, especially minimum temperature of the coldest month, precipitation seasonality, and temperature range, have secondary but also important effects. The species that typically inhabit bark tend to be more abundant when there is a high temperature range. On the other hand, the species that have a clear preference for the aerial litter microhabitat have preference for higher values of precipitation, precipitation of the wettest month, and precipitation of the driest month. Species like *C. apophysatum*, *N. gracile*, and *S. amoeboidea* together or *N. ovatum* and *S. vulgare* have similar niches and appear more frequently together. *T. acutostipes* and *S. pseudoendospora* tend to appear in localities with higher temperatures and higher minimum temperatures of the coldest month and not in aerial litter. *C. apophysatum*, *N. gracile*, and *S. amoeboidea* have certain affinity for bark of living plants and high temperature range. *S. irregulare* and *P. nocturnum* show preference for localities with higher precipitations and lower temperatures, but *P. nocturnum* needs higher values of the minimum temperature of the coldest month.

The GLMs (Table 6) found significant contributions of at least one of the studied variables in all species but *N. ovatum*. The factors with more influence were minimum temperatures of the coldest month, which had negative effects for eight of the species (*C. apophysatum*, *N. gracile*, *P. nocturnum*, *S. amoeboidea*, *S. irregulare*, *S. pseudoendospora*, *S. vulgare*, and *T. acutostipes*) and temperature range having negative effects for four of the species (*P. nocturnum*, *S. amoeboidea*, *S. pseudoendospora*, and *T. acutostipes*). For *S. pseudoendospora* and *S. amoeboidea*, maximum temperature of the warmest month has a positive effect.

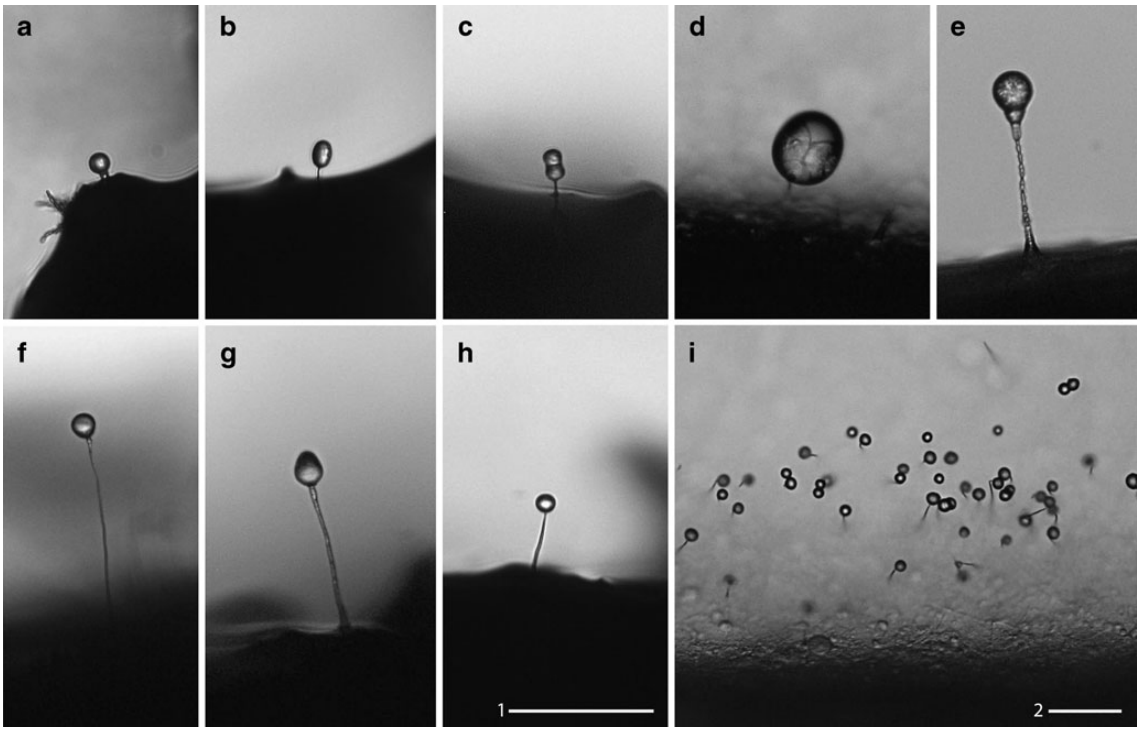

Figure 4 Fruiting bodies of **a** *C. apophysatum*; **b** *E. bisporum* hydrated and **c** dried; **d** *E. oligospora*; **e** *E. zonatum*; **f** *N. gracile*; **g** *N. ovatum*; **h**, **i** *T. acutostipes*. *Bars* 50 μm; *1* for **a**–**h**, and *2* for **i**

Discussion

The methods employed in this paper provide quantitative data and explore for the first time the influence of different climatic variables over protosteloid species in a relatively small area with a Mediterranean climate. The colony-counting method has the advantage of providing a more quantitative approach that makes possible the use of abundance measures and

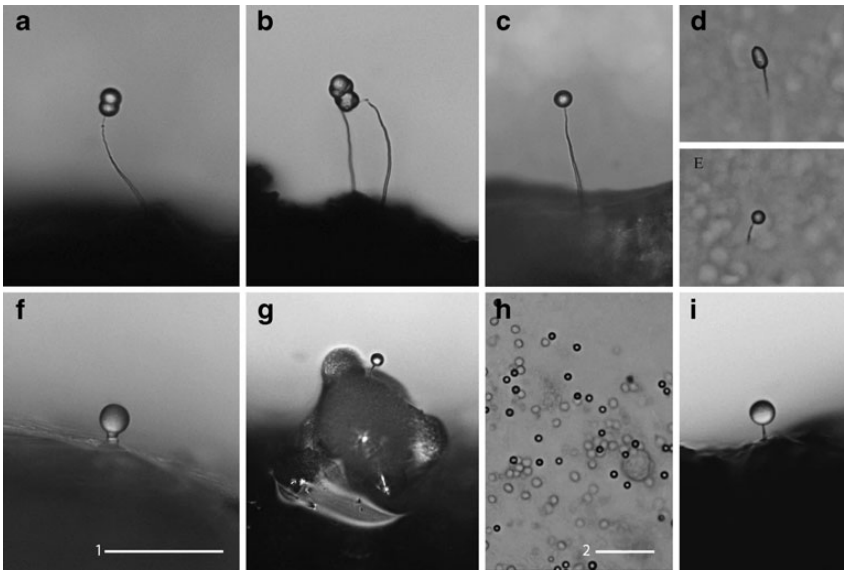

Figure 5 Fruiting bodies of **a**, **b** *P. articulatum*, **c** *P. mycophaga*, **d** *P. arachisporum*, **e** *P. nocturnum*, **f** *S. cavostelioides*, **g** *S. pseudoendospora* fruiting on myxobacteria and **h** in group, **i** *S. amoeboidea*. *Bars* 50 μm; *1* for **a**–**g**, **i** and *2* for **h**

Table 5 Estimates of the total species richness using the abundance-based coverage estimator and rarefaction using the Michaelis–Menten equation as saturation formula

	Species recovered from samples	ACE			Rarefaction		
		Estimate	s.e.	95% confidence interval	Estimate	s.e.	c.c.
A	18	19.6	2.2	18.2, 29.9	18.6	0.3	0.99
G	15	17.8	3.4	15.4, 33.0	16.5	0.5	0.98
B	11	15.5	4.5	11.9, 34.3	15.4	0.1	0.99
Total	18	18.4	0.8	18.0, 22.9	18.6	0.3	0.99

ACE abundance-based coverage estimator, *A* aerial litter, *G* ground litter, *B* bark, *s.e.* standard error, *c.c.* correlation coefficient

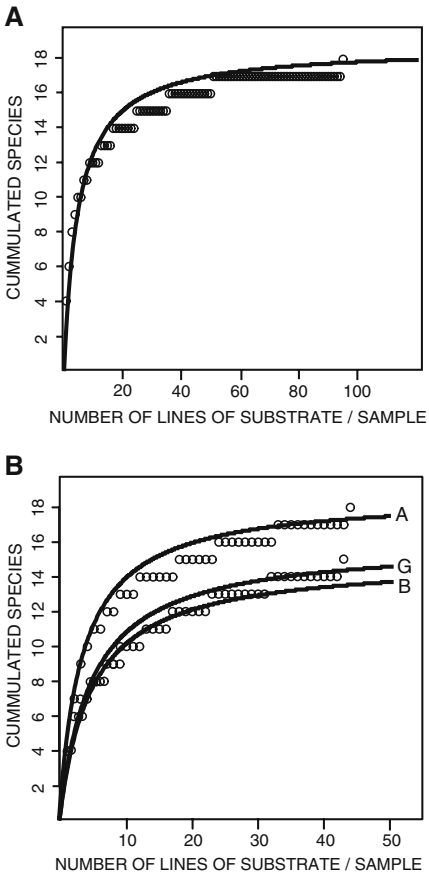

Figure 6 Analysis of the randomly permutated sequence of all samples studied versus cumulative species numbers (*open circles*). These values are the means of 100 runs. The *solid line* shows the results of regression analysis using a saturation function $y = Ax/(B + x)$, where A is the maximum number of species to be expected and B is the number of samples needed to reach half the number of species to be expected. **a** Results for all the samples. **b** Results for the different microhabitats

statistics. As this study is more exhaustive than usual and samples were plated three times, it was an opportunity to emphasize the culturing methodology and optimize the effort needed for this new area. These improvements can be used in the future to generate comparable data sets for the large-scale studies that are necessary for a better understanding of the ecology of these species.

The value of counting colonies vs. counting individual fruiting bodies is that it is an easier, quicker method to carry out and that it is not affected by patchiness of food abundance and allows the use of statistics to test the significance of the observed results. Its main disadvantage could be its subjective component that may have the consequence that results obtained by different observers are not completely comparable. If the method is strictly followed, this source of error can be highly reduced because any inherent errors in the assumption of what a colony is are constant for all samples and all observers. Moreover, this method has the assumption that every colony is originated from an individual propagule that was present on the substrate, but some possible built-in errors are that (1) distinct patches of fruiting that are closer than 2 mm to each other might be separate colonies, also (2) colonies on opposite sides of a piece of substrate may be continuous across the piece of substrate but cannot be seen due to the opacity of the substrate, and (3) two or more colonies could grow together before the first observation could be made.

Another important question is to what extent our results reflect what is actually present in the field. Culture conditions in the lab are different from natural conditions, and this can affect the way propagules germinate and fruit. It is also not well-known if wMY medium has selective effects on the protosteloid organisms and if other enrichments would enable different species to grow. Observed colonies are the result of species that were present in the original samples—at least as propagules—and that were able to germinate, survive, and fruit under culture conditions. In previous studies [17], similar patterns were found on native substrates and on previously sterilized, standardized substrates that were placed in the field and colonized by spores. This suggests that the

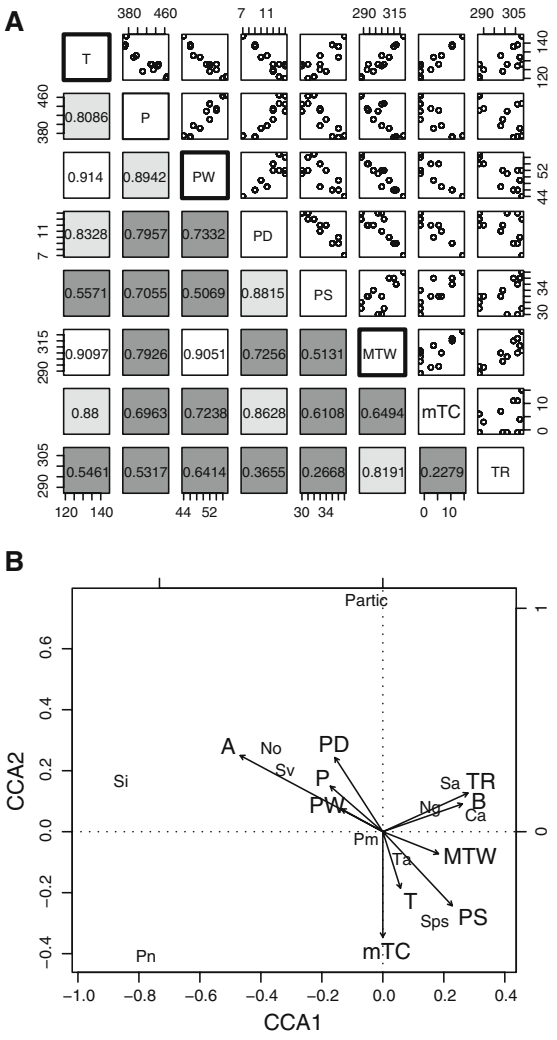

Figure 7 **a** Correlation analyses of all pairs of climatic variables. Each variable name is shown in the intersection between the *row* and the *column* that represent its results. Results from variable pairs are presented in the intersections between *rows* and *columns* of different variables. Plots of all pairs are presented on the *right upper corner*, and their corresponding squared correlation coefficients (r^2) are in the *lower left corner*. Values of r^2 close to 1 and points forming a *line in the plot* indicate high correlation between a pairs of variables. A group of highly correlated variables is *highlighted*. *White*: $r^2>0.9$, *light gray*: $r^2>0.8$, *dark gray*: $r^2<0.8$. **b** Canonical correspondence analysis using abundant, common, and occasional species as dependent variables and climatic and microhabitat variables as independent variables. Each species point in the diagram is at the centroid (weighted average) of the site points in which it occurs, environmental variables are represented by *arrows* that run from the origin to the weights that each variable has in the linear combinations that form the axes *Ca C. apophysatum*, *Ng N. gracile*, *No N. ovatum*, *Partic P. articulatum*, *Pm P. mycophaga*, *Pn P. nocturnum*, *Sa S. amoeboidea*, *Sps S. pseudoendospora*, *Sv S. vulgare*, *Si S. irregulare*, *Ta T. acutostipes*, *P* annual precipitation, *PD* precipitation of the driest month, *PS* precipitation seasonality, *PW* precipitation of the wettest month, *T* mean annual temperature, *MTW* maximum temperature of the warmest month, *mTC* minimum temperature of the coldest month, *TR* temperature range, *A* aerial litter, *B* bark

protosteloid amoebae that are observed in culture are probably, for the most part, the ones that are actively growing and dispersing. The main drawback of using cultures is that there may be differences in the success of propagules from different species in culture, making inter-species comparisons very difficult. Problems with cultures especially affect culture-based quantitative approaches like the colony-counting method because they will underestimate the number of propagules present in the samples and be biased toward the species that germinate and fruit better in culture conditions. This problem will not be solved until quantitative environmental molecular techniques are developed for protosteloid amoebae.

Previous studies about seasonality in protosteloid amoebae [17] show evidence of the existence of cyclic changes in assemblages of protosteloid amoebae where more colonies occurred in samples collected in warmer months and fewer colonies occurred in colder months. What is seen in plates probably reflects a snapshot of what is happening in nature during some period of time just prior to the collection of the samples. If any turnover during seasonal cycles or over longer periods is occurring, it would be missed in present study. To reduce the effect of seasonal differences and get a comparable dataset, samples for this study were collected in October and February, when precipitation and temperature are close to the annual averages. This way it is possible to avoid the direct effect of summer drought and extremely cold temperatures and thus reduce the effect of cyclic changes.

On the basis of the results obtained, it is possible to adapt the culture method to each particular case, depending on the objectives of the research. If the objective is to find as many species as possible or a particular rare species, then using more than one plate per sample is highly recommended. To achieve the ecological objectives of this paper, culturing each sample only once would have provided the best fit between effort and results because only the rarer species would have been missed. Rare species are found in such small numbers that they are strongly affected by random errors, and it is very difficult to use statistics to obtain reliable conclusions about their ecological preferences. For this reason, the ecology of rare species should be studied with a more sensitive method, or with a sampling design specifically oriented which could provide enough raw data to obtain statistically reliable results and minimize errors.

Results presented herein are consistent with previous studies carried out in other temperate areas ([2, 3, 15, 17, 18, 30, 39], see also [21]), and a high percentage of positive samples and number of species per sample were obtained. These studies used different methods and sampling strategies,

Table 6 Results of the generalized linear models for the abundant, common, and occasional species

	Probability distribution	Significant variables in GLM
C. apophysatum	Poisson	(−)PD, (−)mTC**
N. gracile	Quasi-Poisson	(+)P, (+)PS, (−)mTC
N. ovatum	Negative binomial	
P. articulatum	Negative binomial	(−)G*
P. mycophaga	Poisson	(−)B
P. nocturnum	Negative binomial	(−)mTC, (−)TR, (−)G*
S. amoeboidea	Poisson	(+)MTW, (−)mTC*, (−)TR, (−)B*
S. irregulare	Negative binomial	(−)mTC, (−)G
S. pseudoendospora	Poisson	(+)MTW, (−)mTC*, (−)TR, (−)B*
S. vulgare	Negative binomial	(−)mTC
T. acutostipes	Poisson	(−)mTC*, (−)TR, (−)B*

The probability distribution of data and the significant variables are shown. No indication: $p<0.05$

GLM generalized linear models, *P* annual precipitation, *PD* precipitation of the driest month, *PS* precipitation seasonality, *MTW* maximum temperature of the warmest month, *mTC* minimum temperature of the coldest month, *TR* temperature range, *G* ground litter, *B* bark, *(+)* positive effect, *(−)* negative effect

No indication: $p<0.05$, *$p<0.01$; **$p<0.001$

so results have to be compared with caution. In most cases [2, 3, 30, 39], protosteloid amoebae were recorded as presence data on natural substrates. Other approaches were the use of abundance data from standardized substrates [17, 18], or presence data from standardized substrates [15]. The closest area with temperate climate formerly studied, the Somiedo Biosphere Reserve in the northern part of Spain [2], showed a higher species richness. It is remarkable that protosteloid amoebae have a lower species richness in a study area comprised in the Mediterranean region, a biodiversity hotspot for other groups of organisms, in spite of the fact that this study has been more exhaustive. Nonetheless, as the sampling methods were different in each study, observed tendencies should be taken with caution. In order to confirm these results and study their causes in more detail, it would be necessary to perform a new study, including localities from both temperate and Mediterranean regions using the same quantitative method. In all other temperate areas studied, the number of species obtained was lower, except in the Mountains of Northwest Arkansas, USA [5] and Great Smoky Mountains National Park, USA [30]. In the tropics and high-latitude areas that have been previously surveyed, species richness was also lower than in this study, being higher only in forests from Malawi and Kenya [21] and Puerto Rico and Hawaii [38].

It has been previously observed that it is possible to find more differences in assemblages of species from different microhabitats in the same locality, than when comparing samples from the same microhabitat collected in different localities [18, 34]. However, species composition and relative abundance also vary in each microhabitat at different latitudes [21]. The influence of various climatic factors over species found in this study area has been studied using CCA, an ordination method that considers all species together to find the ecological variables that maximize the differences between their niches, and GLM, a parametric method that studies each species individually to find out its requirements. In the CCA, the microhabitat variables were the most important for differentiating the niches of the studied species, and the climatic variables had a secondary but also important effect, but all the variables studied only explain a 15.92% of variation in the data. The incorporation of other sources of information like biotic interactions, pH, concentration of nutrients, and controlling the effect of covariates may improve the quality of future models.

Aerial litter was the microhabitat in which more species were found, and it had the highest abundance of protosteloid amoebae, a result that was obtained in most works carried out in similar latitudes [2, 18, 39]. It was also the microhabitat with the highest evenness, suggesting that species living in this microhabitat may tolerate wider ranges of climate change or that this microhabitat is less heterogeneous than others. According to CCA, aerial litter microhabitat has significant effects on niche segregation, and the species with a clear preference for this microhabitat tend to be more abundant in localities with higher precipitation, lower temperatures of the warmest month, and they usually can tolerate lower values of minimum temperature of the coldest month. This result is also consistent with results obtained in studies made in high latitudes [20, 35]. In this kind of habitats, temperatures are low and precipitation is usually high, and most protosteloid species found are those typical of aerial litter in temperate areas.

Results from the CCA also show that the species that are typical bark inhabitants tend to be more abundant if there is a high temperature range and low annual precipitation. Bark species are usually more abundant in arid grasslands and desert ecosystems, where precipitations are low and there is a high contrast of temperatures, but in this kind of habitats are found fewer protosteloid amoebae common on dead aerial plant parts [34]. In the rarefaction analysis, this microhabitat's curve was less steep than the others, indicating that bark species were less evenly distributed in the samples.

Results obtained with GLM gave further information about the individual preferences of the species and the influence of the climatic factors studied. The problem is that the area studied is too small to have a wide sampling of the environmental conditions that the species can tolerate, so these tendencies cannot be reliably extrapolated out of this area. All species but *P. articulatum* and *P. mycophaga* show preference for localities with lower minimum temperatures of the coldest month. This variable was also significant in the CCA, and it seems to have a very important effect on protosteloid species. *N. gracile*, a species usually more common in tropical latitudes, seems to prefer higher annual precipitation and precipitation seasonality. For *S. pseudoendospora* and *S. amoeboidea*, high maximum temperature of the warmest month has a positive effect.

When comparing relative abundances of protosteloid amoebae obtained in other studies carried out in temperate areas, some differences arise, but most results are concordant with those in this study. However, comparisons between studies made so far are merely informal observations that can be used as a starting point for further work. Two abundant species in this study, *P. mycophaga* and *S. pseudoendospora*, were also abundant in all other studies in temperate areas and usually abundant or common in tropics and high latitudes. They are expected to be a major part of any biota of protosteloid amoebae [21]. *S. amoeboidea*, abundant in present study, was abundant in the Ozark Mountains of Northwest Arkansas, USA [5], in the Somiedo Biosphere Reserve [2], and in one study from tropical areas [21]. It is a widespread species but its abundance varies from locality to locality without a clear pattern. *T. acutostipes*, a species usually more abundant in temperate localities than in the tropics, was also abundant in [30] and common in the Somiedo Biosphere Reserve [2]. It is remarkable that *C. apophysatum* was a common species here. This species is usually rare or occasional in temperate areas, but it is a common or abundant species in tropical areas [21]. In the Somiedo Biosphere Reserve [2], it was an occasional species. Another interesting anomaly is that *S. irregulare* is an occasional species here. It is an abundant species in most studies in temperate areas [21], except in [29] where it is occasional. *P. articulatum*, which was not recovered from samples from Somiedo, is moderately abundant here. This species is more commonly encountered in drier habitats worldwide and has been traditionally considered a bark inhabiting species [36]. It is interesting that, here, this species was found in microhabitats other than bark, especially in aerial litter. It is also remarkable that *N. gracile*, usually a species with preference for ground litter [36], shows more preference for aerial litter in this study area. However, results about *N. gracile* may not be completely reliable because this species cannot be distinguished from *Ceratiomyxella tahitiensis* on the basis of fruiting body morphology, so it is likely we observed both of those species and one might have more preference for aerial litter than the other.

Our present results and our earlier results from Somiedo [2] confirm the excellence of Spain as study area for protosteloid amoebae. The qualitative differences in the occurrence of protosteloid amoebae in the two studies lead us to believe that comparison of their communities in the different ecoregions of Spain may prove to be useful for understanding the biogeography of these organisms in general. Just as the Mediterranean climate seems to be rich in other mycetozoans [12, 13, 26], it is rich in protosteloid amoebae. Thus, the Mediterranean climatic region of Spain can be used as a baseline for comparison with the protosteloid amoebal communities of other Mediterranean regions of the world. The use of these quantitative methods can serve as a blueprint for other studies to test and compare relative abundances of protosteloid species between areas and microhabitats, and the optimization of the sampling method that has been carried out can help to increase the effectiveness of ecological studies in this interesting bioregion. Using these methods, it will be possible to understand the influence of environmental factors on this group and compare its pattern to both those of other microorganisms and of multicellular organisms. The study of microhabitat conditions and their relationship with major climatic factors is a stepping stone for understanding both small- and large-scale distribution of this kind of organisms.

Acknowledgments We wish to thank Eva García Carvajal for helping in field work, Fátima Durán for technician work, Diana Wrigley de Basanta for reviewing the style of the text, and Lora Lindley Shadwick, John Shadwick, Matt Brown, George Ndiritu, and Carlos Rojas for all their kind suggestions. This work has been supported by the Research Project (CGL2005-00320/BOS and CGL2008-00720/BOS) of the Ministry of Science and Innovation of Spain and the National Science Foundation grants DEB-0316284 (PBI) and DEB-0329102 (PEET).

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Capítulo 3: Ecología de los Protostélidos Ibéricos

Tras establecer un método de muestreo y cultivo apropiados, se recolectaron nuevas muestras en el noreste de la Península. En esta etapa del trabajo comprobamos que el tiempo que era necesario invertir en cada cultivo hacía inviable estudiar la Península Ibérica completa con la intensidad necesaria en el tiempo disponible, por lo que optamos por completar el muestreo en forma de transecto diagonal hacia el suroeste de la Península. Este transecto recorre zonas con clima de montaña, eurosiberiano, zonas de transición del eurosiberiano al mediterráneo, semiárido, un clima mediterráneo más continentalizado, y clima mediterráneo con influencias marítimas.

Los datos obtenidos sobre las abundancias de las especies se analizaron para comprobar si a la escala de la Península Ibérica podían detectarse ya los efectos del clima sobre la abundancia de las especies. El siguiente paso fue estudiar cómo actúa el clima sobre las comunidades de protostélidos presentes en cada microhábitat y, tras comprobar que los protostélidos no se distribuyen al azar sino que siguen ciertos patrones dependientes del clima en estas zonas, se intentó averiguar cuáles son las preferencias individuales de cada especie. Finalmente, los datos disponibles de presencia de las especies se usaron junto con las variables climáticas para elaborar modelos de nicho ambiental que constituyen predicciones de la probabilidad de encontrar las especies usando la misma metodología, y que podrán servir de ayuda para diseñar futuros muestreos y como hipótesis de partida en estudios biogeográficos. Los resultados obtenidos se presentan en el siguiente artículo:

Aguilar M, Lado C. (2012). Ecological Niche Models reveal the importance of variability in climatic conditions for the biogeography of protosteloid amoebae. ISME Journal (in press).

Resumen: La disponibilidad de hábitats y las preferencias ecológicas de las especies se encuentran entre los factores más importantes para determinar el éxito de los procesos dispersivos y por tanto para dar forma a la distribución de los protistas. Hemos explorado las diferencias en los nichos fundamentales y las distribuciones potenciales de un gremio ecológico de hongos mucilaginosos – las amebas protosteloides – en la Península Ibérica. Un conjunto de muestras recolectadas en un transecto de aproximadamente 1000 km desde el noreste hasta el suroeste de la Península fue usado para testar la hipótesis de que, junto con la existencia de hábitats apropiados, las condiciones climáticas pueden determinar la probabilidad de supervivencia de las especies. Aunque las amebas protosteloides

comparten morfologías y modos de vida similares, los análisis de correspondencia canónica mostraron que tienen distintos óptimos ecológicos. Mediante modelos de nicho ambiental de Maxent se realizaron predicciones de la probabilidad de presencia de las especies en áreas que no han sido muestreadas todavía, y dichos modelos fueron usados para generar mapas de distribución potencial que también se compararon. Los factores climáticos más importantes fueron en ambos análisis las variables que miden cambios en las condiciones a lo largo del año, confirmando que la alternancia de cuerpos fructíferos, quistes y estados ameboides en los ciclos de vida constituyen una ventaja para sobrevivir en un ambiente cambiante. La afinidad por los microhábitas parece estar influenciada por factores climáticos, lo que sugiere que las condiciones microambientales podrían variar a escala local y cambiar junto con el clima externo a una escala mayor.

NOTA: El material suplementario correspondiente a este capítulo se encuentra en el Apéndice 1 situado al final de la memoria.

Ecological Niche Models reveal the importance of variability in climatic conditions for the biogeography of protosteloid amoebae

María Aguilar & Carlos Lado

Habitat availability and environmental preferences of species are among the most important factors in determining the success of dispersal processes and therefore in shaping the distribution of protists. We explored the differences in fundamental niches and potential distributions of an ecological guild of slime moulds – protosteloid amoebae - in the Iberian Peninsula. A large set of samples collected in a north-east to south-west transect of approximately 1000 km along the peninsula was used to test the hypothesis that, together with the existence of suitable microhabitats, climate conditions may determine the probability of survival of species. Although protosteloid amoebae share similar morphologies and life history strategies, canonical correspondence analyses showed that they have varied ecological optima, and that climate conditions have an important effect in niche differentiation. Maxent environmental niche models provided consistent predictions of the probability of presence of the species based on climate data, and they were used to generate maps of potential distribution in an "everything is everywhere" scenario. The most important climatic factors were, in both analyses, variables that measure changes in conditions throughout the year, confirming that the alternation of fruiting bodies, cysts and amoeboid stages in the life cycles of protosteloid amoebae constitutes an advantage for surviving in a changing environment. Microhabitat affinity seems to be influenced by climatic conditions, which suggests that the micro-environment may vary at a local scale and change together with the external climate at a larger scale.

Introduction

General biogeographic patterns of free-living protists are still a subject of debate. The "everything is everywhere" hypothesis states that most free-living protists have huge population numbers and a small body size, which may cause high rates of dispersal and a low rate of allopatric speciation and endemism (Finlay & Clarke, 1999; Finlay, 2002; Finlay et al, 1999, 2001; Finlay & Fenchel, 2004). Therefore, the individual environmental preferences of the species and habitat availability would be major forces in shaping their distributions (Finlay, 2002; Fenchel & Finlay, 2006). On the other hand, there is also evidence of limited dispersion and geographically restricted organisms (Foissner, 2006; Smith & Wilkinson, 2007; Foissner et al, 2008; Vanormelingen et al, 2008), which are consistent with a "moderate endemicity" scenario.

The fundamental niche of a species is the set of environmental conditions that make possible its long-term survival (Hutchinson, 1957), excluding the effect of biotic interactions, restricted dispersion, or human influence, that can prevent the species from inhabiting all the areas encompassing its full ecological potential (Pulliam, 2000; Anderson & Martínez-Meyer, 2004). Using ecological niche modelling techniques, it is possible to devise a model of a species' environmental requirements from the conditions of sites of known occurrence, obtaining a mathematical function that represents its fundamental niche. Results can later be projected into new areas with known characteristics to predict the proba-

bility of presence of the species there and trace their potential distributions (Phillips et al, 2006).

In this paper we explore the differences in fundamental niches and potential distributions of protosteloid amoebae in an area of the south-west of Europe, the Iberian Peninsula. Protosteloid amoebae, formerly known as protostelids, constitute an ecological guild of slime moulds that are scattered within the amoebozoa tree (Shadwick et al, 2009b, Supplementary Figure S1), and act as predators of decomposers of decaying plant tissues (Spiegel, 1986; Spiegel et al, 2007). All protostelid species have in common that they are amoeboid organisms with the ability, under certain conditions, to produce microscopic fruiting bodies (Olive, 1975; Spiegel, 1986; Spiegel et al, 2007), which consist on one to a few spores at the tip of a delicate acellular stalk. They also produce various trophic stages that range from amoebae or amoeboflagellates to microscopic plasmodia. Available data on the ecology and distribution of these organisms show evidence that compositional differences exist between microhabitat types at a local scale (Moore & Spiegel, 2000b, 2000c; Moore et al, 2000; Shadwick & Stephenson, 2004; Powers & Stephenson, 2006; Aguilar et al, 2007; Kosheleva et al, 2009). Microhabitats are small, localized habitats within a larger ecosystem and have their own environmental characteristics that presumably are more or less constant across areas with similar climates. On a continental scale, is it also possible to find different communities in the same microhabitat, caused by different climate conditions (Ndiritu et al, 2009; Aguilar et al, 2011). Thought not statistically tested, there is a tendency that at least some species move towards ground litter in boreal areas (Spiegel & Stephenson, 2000), and towards aerial litter in tropical areas (Moore & Spiegel, 2000c).

Approximately 80% of the surface of the Iberian Peninsula has a Mediterranean climate. This climate is characterized by warm to hot, dry summers and mild to cool, wet winters. There is always a summer drought caused by subtropical high-pressure cells, that make rainfall very unlikely except for occasional thunderstorms, and

Figure 1 – Map of the Iberian Peninsula with selected localities for the analyses. Localities are represented as black circles, and numbers correspond to information in Supplementary Tables 2, 3.

almost all precipitation falls during the colder months of the year. In Mediterranean regions that are in the proximity of the sea, temperatures are generally moderate with a comparatively small annual temperature range, although the daily range of temperature in the summer is usually large. Regions further from the coastal areas have a lower temperature in the winter and high annual temperature ranges (Agencia Estatal de Meteorología, 2011; Walter, 1984; Di Castri et al, 1981). On the other hand, areas with an Oceanic climate, that cover the remainder 20% of the peninsular area and are located on the northern coastal strip, have moderately cool summers and warmer winters than in the inland areas, with a narrow annual temperature range. They lack a dry season and precipitation is evenly dispersed through the year.

The objective of this study was to explain the geographical patterns of protosteloid amoebae in the Iberian Peninsula from an ecological point of view. For this, we used a large number of samples collected along a Northeast to Southwest diagonal transect, with comparable methodology. Maxent environmental niche models and canonical correspondence analyses (CCA) were used to evaluate the hypothesis that ecological niches of protosteloid species strongly collaborate in shaping their geographic distribution, and that once their ecological preferences are known, it is possible to predict their probability of occurrence in other similar areas not yet studied. Both climatic variables and microhabitat were included in CCA, in order to evaluate and compare their effects.

Materials and Methods

Sampling

Results presented in this study were obtained by the analysis of data collected in the Iberian Peninsula during years 2005-2009, along a diagonal transect of approximately 1000 km from the Northeast to the Southwest of the peninsula. At each site, samples were collected within a radius of approximately 20 m. We aimed to collect 10 samples from different plant species in each locality for subsequent laboratory culture, but it was not possible in all cases due to the absence of suitable tissues. The 10 samples belonged to different microhabitats and were distributed as follows: 4 samples of aerial litter (assemblage of dead but still attached parts of standing plants), 4 samples of ground litter (the layer of twigs, leaves, and other plant debris extending over the soil surface), and 2 samples of bark of living plants. Collected samples were placed in individual paper bags and air-dried in the laboratory. They were cultured as described elsewhere (Aguilar et al, 2011), and identified on the basis of fruiting body morphology. Species abundances were quantified following the methods based in colony counts described in Aguilar et al (2011).

Database

The number of colonies from each species in each sample was recorded in a database, also containing microhabitat type and spatial coordinates of the localities. To improve the quality of the models, data already published in Aguilar et al (2007, 2011) were also included. This database was refined to avoid excessive differences in sampling effort that may bias the results. Sampling sites were projected on a geographic information system (GIS), and points were randomly eliminated from oversampled areas. The points finally selected (Figure 1) were associated with data from a total of 23 species. With this database already refined, two matrices were built, for use in the analyses later. The first matrix contained

presence data of the species that were present in at least 10 localities (Supplementary Table S2). The second matrix included abundance data of species in different microhabitats at each site (Supplementary Table S3), but as in some cases the samples were cultured more than once (see Aguilar et al, 2011), culturing effort differences in samples were corrected by dividing the number of colonies between the number of cultures and rounding down results. A third matrix was constructed with values of the 19 Bioclim variables from the WorldClim database (Hijmans et al, 2005) (www.worldclim.org, March 2011) in current conditions with a 30 arc-seconds resolution for each selected locality, that were extracted with Spatial Analyst extension of ArcGIS (Supplementary Table S4).

Maxent

The matrix with presence data was analysed with the program Maxent version 3.3.3e, March 2011 (Phillips et al, 2006; Phillips & Dudik, 2008). Niche models for the species present in at least 10 localities (14 species) were calculated with Bioclim variables from WorldClim in current conditions with a 30 arc-seconds resolution. Preliminary models were developed using all 19 variables. To prevent over-fitting, the variables that were considered to contribute less to the model were removed after observing the estimates of their relative contributions, and the jacknife tests of variable importance implemented in the Maxent software. Finally, the models were run with selected variables only, and with 80% of the occurrence localities as training data, reserving the remaining 20% for testing results. Models were evaluated based on receiver operating characteristic (ROC) analysis, that generates the AUC (area under the curve) score.

Canonical Correspondence Analyses

Correlation between all pairs of climatic variables in the matrix with extracted values from Bioclim at each point, was studied using regression analyses in R 2.12.2 (R Development Core Team, 2008) (Supplementary Table S5), and highly correlated variables without a clear biological significance in a the study area considered - mean temperature of the warmest quarter, precipitation of the driest month, precipitation of the wettest quarter and precipitation of the driest quarter – were removed. A stepwise canonical correspondence analysis (stepwise CCA) was performed with R 2.12.2 and the vegan package (Oksanen et al, 2008). This analysis sequentially removes the least important variables, and thus makes possible to distinguish which variables contribute more to differentiate the niches of the species. Species were scaled proportional to eigenvalues, sites were unscaled (weighted dispersion equal on all dimensions), and permutation tests were carried out.

Mantel tests

Geographic distances between sampling points, and Bray-Curtis dissimilarity between assemblages were measured using the packages fields (Nychka, 2007) and ecodist (Goslee & Urban, 2007) in R. Ecological distance between localities was calculated as an Euclidean distance in a multidimensional space determined by the 19 Bioclim variables previously centered and scaled. Mantel tests and partial mantel tests were performed with vegan, using 1000 permutations, and Pearson product-moment correlation coefficient.

Results

Maxent

Variables selected for each species model and their relative contributions are

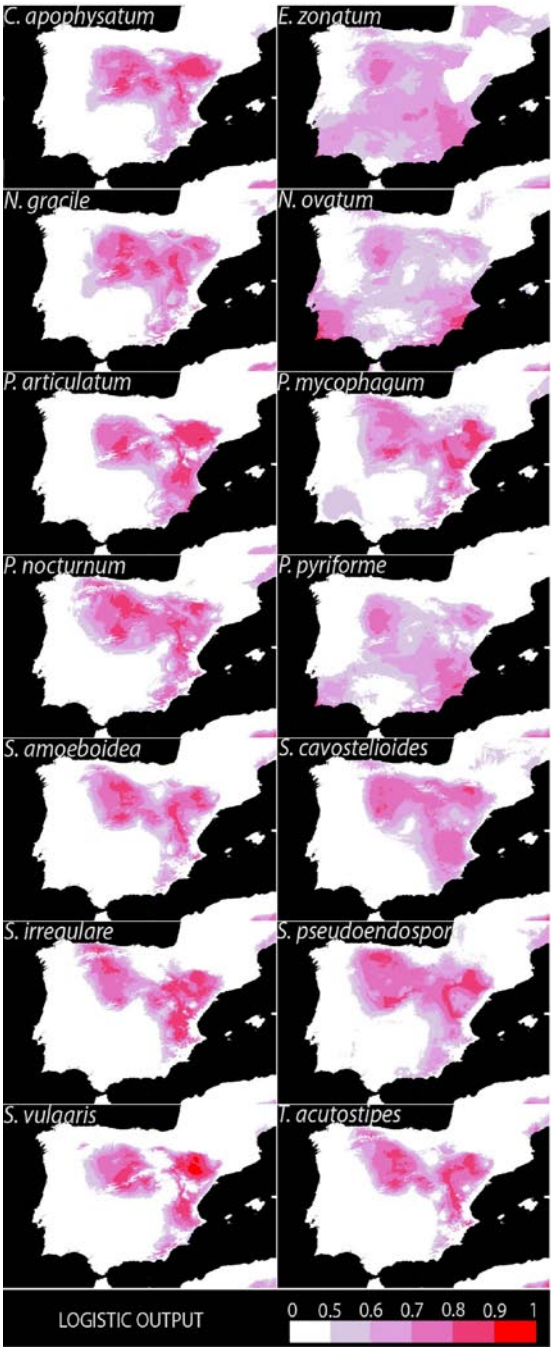

Figure 2 – Predictive ecological models based on the Maxent algorithm of the species of protosteloid amoebae with more than 10 occurrences. Probabilities of presence >0.5 are represented using different colour shades.

shown in Table 1. All models had high AUC scores (> 0.9) for both training and test data. Environmental variables that most frequently had a high percentage contribution to the models were isothermality, mean diurnal range, precipitation of the coldest quarter, temperature seasonality, precipitation of the warmest quarter, and precipitation seasonality.

Projected models are displayed in Figure 2. Most species prefer the inland areas of the northern half of the peninsula and the eastern coastal strip. All these areas are characterized by low annual precipitation (generally less than 600 mm) and low precipitations even in the winter (ranging 65- 145 mm in the coldest quarter of the year), but higher precipitations in the summer than in other Mediterranean areas of the peninsula (50-100 mm). Temperatures are relatively cold (annual means ranging 9.5- 14.5) and vary throughout the year (standard deviation 5.5-6.7).

But some species, namely *Protostelium mycophagum*, *Endostelium zonatum*, *Nematostelium ovatum* and *Protostelium pyriforme*, can also tolerate areas in the Southwest, with higher annual mean temperatures (14-18ºC) and a lower temperature seasonality (standard deviation 3.6-5.2). These areas are also characterized by a higher precipitation in the winter than in other Mediterranean areas in the Iberian Peninsula (150-250 mm in the coldest quarter of the year), and a severe summer drought (precipitation <50 mm in the warmest quarter of the year). Some other species, like *Schizoplasmodiopsis vulgaris*, *Schizoplasmodiopsis pseudoendospora*, *Protosporangium articulatum*, *Soliformovum irregulare* and *Cavostelium apophysatum*, have large areas predicted with very high probability (0.8-0.9) in the north-east of

the peninsula, in an area with very low annual precipitation (140-160 mm), and low precipitation both in colder (<70 mm) and warmer months (70-100 mm), moderately high annual mean temperature (13-16 ºC), with relatively high temperature seasonality (standard deviation 6-6.5).

Canonical Correspondence Analyses

After the stepwise process, the independent variables that were not removed by the analysis and were used to generate the final ordination, were Bioclim's annual mean temperature, isothermality, precipitation seasonality, precipitation of warmest quarter, precipitation of coldest quarter, aerial litter microhabitat, ground litter microhabitat, and bark microhabitat.

The final CCA obtained (Figure 3) had a total inertia of 3.1527, a constrained inertia of 0.5522 (proportion 17.52%), and an unconstrained inertia of 2.6005 (82.48%). The permutation test for the axes was significant (p=0.005), and the permutation test for the independent variables showed that isothermality (p=0.010), precipitation seasonality (p=0.055), precipitation of warmest quarter (p=0.005), precipitation of coldest quarter (p=0.005), aerial litter microhabitat (p= 0.005), and ground litter microhabitat (p=0.080), and bark microhabitat (p=0.05) had significant effects.

The species that have preference for ground litter and bark microhabitats in this areas – *Schizoplasmodiopsis pseudoendospora*, *Nematostelium ovatum*, *Nematostelium gracile*, *Schizoplasmodium cavostelioides*, *Schizoplasmodiopsis reticulata*, *Endostelium amerosporum*, *Cavostelium apophysatum* and *Schizoplasmodiopsis amoeboidea* – are more abundant where annual mean temperature is high, and precipitation of the coldest quarter is low. On the other hand, the species that typically inhabit aerial litter here – *Protostelium mycophagum*, *Protostelium pyriforme* and *Protosporangium bisporum* –, are more frequently identified in localities with lower annual mean temperature, and higher precipitation seasonality.

There is a group of species with affinities for areas with high isothermality and precipitation seasonality but low precipitations both in colder and warmer months – *Tychosporium acutostipes*, *Soliformovum irregulare*, P*rotosporangium articulatum* and *Schizoplasmodiopsis vulgaris*. By contrast, another group of species shows clear preference for high precipitation of the warmest quarter values and low precipitation seasonality and isothermality – *Microglomus paxillus*, *Protostelium nocturnum*, *Echinostelium bisporum*, *Echinosteliopsis oligospora*, *Endostelium zonatum* and *Protostelium arachisporum*.

Mantel tests

Bray-Curtis dissimilarities between protosteloid assemblages were less correlated with geographic distance ($r = 0.1464$, $p = 0.003$), than with ecological distance ($r = 0.2453$, $p < 0.001$). Using partial mantel tests, Bray-Curtis dissimilarities were even less correlated with geographic distance when removing the effect of ecology ($r = 0.0291$, $p = 0.256$). On the other hand, the correlation between Bray-Curtis dissimilarities and ecological distances did not significantly decrease after removing the effects of geographic distance ($r = 0.201$, $p < 0.001$).

Discussion

How does the environment select? Modelling fundamental niches

A better understanding of the ecology and dispersal mechanisms of protists is

	Localities	Variables																		AUC training	AUC test
		AM	DR	IT	TS	MTW	mTC	AR	TWeQ	TDQ	TWQ	TCQ	AP	PWe	PD	PS	PWeQ	PDQ	PWQ	PCQ	
C. apophysatum	42 (34, 8)		14.4	41.8	10		3.7		3.3									2.5	10.9	13.4	0.959	0.967
E. zonatum	13 (11, 2)			21.5						68		0.9					9.5		0.1		0.908	0.931
N. gracile	38 (31, 7)		23	48.4	10.1		0.7		5.2						2.2	1.9	0.5		0.7	7.-1	0.967	0.991
N. ovatum	14 (12, 2)			69.4	2.2												9.2		19.3		0.933	0.931
P. articulatum	20 (16, 4)		6.1	33.9	8.1		0.1			0					5		3.7		23.7	19.5	0.966	0.992
P. mycophagum	66 (53, 13)		12.6	57.2	10				2.4	1			3.2			3.6		1.4	1.6	7	0.969	0.974
P. nocturnum	30 (24, 6)		15.8	53.6	10.4		2.2		5.3			1.1			1.1	4.3			1.1	5.1	0.956	0.93
P. pyriforme	15 (12, 3)		15.1	30.3						11.4							0		31.9	11.3	0.941	0.952
S. amoeboidea	47 (38, 9)		21.8	50.6	7.7				2.2						4.4	4.8			0.6	7.8	0.972	0.984
S. cavostelioides	16 (13, 3)			57.1	1.1							1.2				11.2	0		18.3	11.2	0.946	0.967
S. irregulare	30 (24, 6)		10.3	55.5			3.2		3						0	6.9		10.4	0.8	9.8	0.972	0.985
S. pseudoendospora	59 (48, 11)	0.3	14.8	57.7	11.2				1.5	0.4						5.7		2.7	2.5	3.3	0.96	0.969
S. vulgaris	27 (22, 5)		12.5	34	9		2.3			1.5					7.3		4		19	30.3	0.969	0.978
T. acutostipes	48 (39, 9)		20.1	47.6	12.6		0.2		1.9						3.8	7.2				6.7	0.971	0.958

Table 1 - Results of the Maxent Niche Models. The table gives the estimated percentage of relative contribution of selected Bioclim variables for each species' final model, and the area under the receiver operating characteristic (ROC) curve for training and test data. The 50% variables with higher contributions for each species are highlighted in blue. Localities: total number (training, test), AM: annual mean temperature, DR: mean diurnal range, IT: isothermality, TS: temperature seasonality, MTW: maximum temperature of the warmest month, mTC: minimum temperature of the coldest month, AR: temperature annual range, TWeQ: mean temperature of the wettest quarter, TDQ: mean temperature of the driest quarter, TWQ: mean temperature of the warmest quarter, TCQ: mean temperature of the coldest quarter, AP: annual precipitation, PWe: precipitation of the wettest month, PD: precipitation of the driest month, PS: precipitation seasonality, PWeQ: precipitation of the wettest quarter, PDQ: precipitation of the driest quarter, PWQ: precipitation of the warmest quarter, PCQ: precipitation of the coldest quarter, AUC training: area under the ROC curve for training data, AUC test: area under the ROC curve for test data.

essential for interpreting their biogeographical patterns. In small organisms with an efficient dispersion, the availability of an appropriate habitat would be the principal filter for their establishment on a new area. Knowing the requirements of a species it would be possible to check if propagules can reach all potentially suitable habitats or, on the contrary, their dispersion has been limited in some directions.

The size range of protostelid spores (ca. 4-50 μm in diam.) gives them the potentiality to be easily dispersed, which justifies to use the "everything is everything" model as an approximation to what is happening to these organisms. Assuming that the distribution of the species is not hindered by geographic constraints, as the results of the Mantel tests confirm, the probable distribution of the species was extrapolated based on the climatic data of the sites where they were found.

When constructing predictive models of the niche of a species, the goal is to predict which areas form part of its potential distribution (Anderson & Martínez-Meyer, 2004). To create a satisfactory model, it is very important to make a cautious sampling design to get a sufficiently representative sample. Our sampling design attempted to balance three objectives. One objective was to cover a wide sampling area with different climates to have a bigger picture of the species' ecology, and also to address the dispersal efficiency of protostelids at this scale. Another was to know each locality in sufficient detail. And finally, the third objective was to equalize the effort along the whole transect. But for interpreting the results, we must keep in mind the limitations of our models. As a consequence of the sampling strategy, the generated models do not represent predictions of presence/absence of the species in absolute terms, but provide an estimate of the probability of finding protosteloid amoeba in Mediterranean areas of the Iberian Peninsula using the same methodology and effort.

Our results show that, despite that protosteloid amoebae are considered to share similar morphological characteristics and life history strategies, their environmental niches are not completely the same and each species has its own climatic and microhabitat preferences, confirming results obtained in Aguilar et al (2011) with a smaller data set. Differences in climatic conditions cause the species composition and the structure of the assemblages to vary from locality to locality, being this influence stronger than the effects of geographic distance. These results are also supported by previous studies which, although do not deal with the influence of climatic variables on protostelid assemblages, show that there are differences in species composition when comparing areas with temperate (Aguilar et al, 2007; Best & Spiegel, 1984; Brown & Spiegel, 2008; Moore & Spiegel, 1995; Moore & Spiegel 2000a, 2000b; Shadwick & Stephenson, 2004; Shadwick et al, 2009a; Tesmer et al, 2005), tropical (Moore & Spiegel, 2000c; Moore & Stephenson, 2003; Ndiritu et al, 2009; Powers & Stephenson, 2006; Stephenson et al, 1999), and boreal climates (Moore et al, 2000; Spiegel & Stephenson, 2000; Kosheleva et al, 2009).

Influence of the climate

For the elaboration of the Maxent models and CCA all the Bioclim variables were initially included, but final models were constructed after removing less informative climatic factors. All 19 Bioclim variables were considered in preliminary Maxent models, and the least important

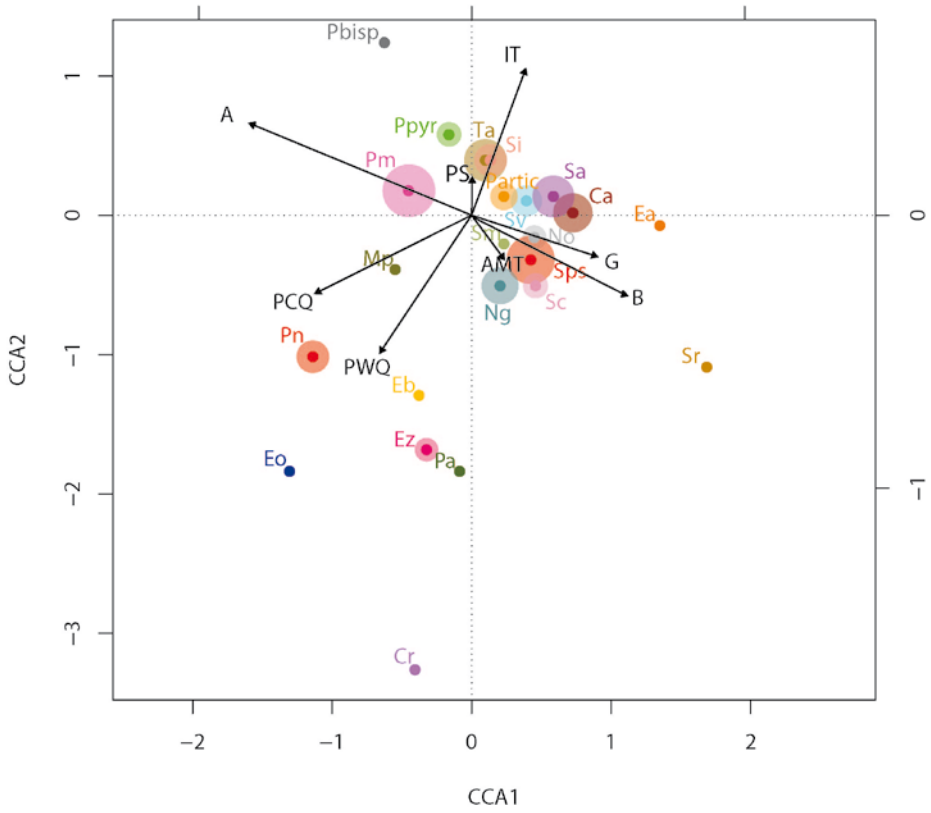

Figure 3 – Canonical Correspondence Analysis (CCA) using species as dependent variables and climatic and microhabitat variables as independent variables. Each species point in the diagram is at the centroid (weighted average) of the site points in which it occurs. Environmental variables are represented by arrows that run from the origin to the weights that each variable has in the linear combinations that form the axes. A: aerial litter, G: ground litter, B: bark, AM: annual mean temperature, IT: isothermality, PS: precipitation seasonality, PCQ: precipitation of the coldest quarter, PWQ: precipitation of the warmest quarter, Ca: *Cavostelium apophysatum*, Cr: *Clastostelium recurvatum*, Ea: *Endostelium amerosporum*, Eb: *Echinostelium bisporum*, Eo: *Echinosteliopsis oligospora*, Ez: *Endostelium zonatum*, Mp: *Microglomus paxillus*, Ng: *Nematostelium gracile*, No: *N. ovatum*, Partic: *Protosporangium articulatum*, Pbisp: *P. bisporum*, Pa: *Protostelium arachisporum*, Pm: *P. mycophagum*, Pn: *P. nocturnum*, Ppyr: *P. pyriforme*, Sa: *Schizoplasmodiopsis amoeboidea*, Sm: *S. micropunctata*, Sps: *S. pseudoendospora*, Sr: *S. reticulata*, Sv: *S. vulgaris*, Sc: *Schizoplasmodium cavostelioides*, Si: *Soliformovum irregulare*, Ta: *Tychosporium acutostipes*.

were subsequently removed to prevent possible over-fitting artifacts. For the CCA, preliminary pairwise regression analyses were used to evaluate correlation and to remove highly redundant variables that were considered as less informative in a Mediterranean/Oceanic climate. After that, the analysis was run in several steps, which sequentially removed the variables with a lower contribution. It is remarkable that the climatic factors selected were in both cases variables that measure changes in conditions throughout the year, like isothermality, temperature ranges, precipitation seasonality, and precipitation of the coldest and warmest quarters. Protosteloid amoebae seem to have the ability to resist variations in their environment, as their peculiar morphology suggests. Spores can survive for a long time and during prolonged pe-

riods of drought (Kosheleva et al, 2009), and their life cycles, that alternate stalked fruiting bodies with trophic stages that vary from amoeboflagellates to nonflagellated amoebae, reticulate plasmodia, and cysts (Olive, 1975; Spiegel, 1986), seem particularly suited to make possible their survival with changing external conditions. The CCA results (Figure 3) suggest that what differentiates the climatic niches of the species is precisely the type of change - temperature, precipitation or both - and the magnitude of change that can be tolerated by each of them.

Unfortunately, previous information about the ecology of these organisms is scarce and all available data have been obtained with various methodologies that are not necessarily comparable. Also, results obtained in this study cannot necessarily be extrapolated to a worldwide scenario, and tendencies may be different in other major climates. Therefore comparisons between studies must be made with caution. Maxent, based on known presence data, predicts the ecological conditions in which it is likely that a species can survive, and CCA uses abundance data to represent the species ecological optima as points in a new coordinate system which maximizes their differences. Thus Maxent models give us the opportunity to compare the similarities between species, while CCA is a powerful tool to analyze their differences.

According to Maxent models (Figure 2), most protosteloid species can be easily found in inland areas located in the northern half of the Iberian Peninsula and the eastern coast, with mild annual mean temperatures, a moderate annual temperature range and milder drought periods. But some species show also a greater tolerance for more extreme variation. A group of species - *Protostelium mycophagum*, *Endostelium zonatum*, *Nematostelium ovatum*, and *Protostelium pyriforme* - has a higher tolerance to areas with higher temperatures with little variation along the year, and a lower precipitation in summer. This species seem to be evolutionary unrelated (Spiegel, 1986; Shadwick et al, 2009b, Supplementary Figure S1), and little is known about their ecology in other areas. Most of them have been reported to show preferences for tropical and temperate areas. *Protostelium mycophagum* is usually a very abundant species worldwide, and it is probably a generalist with a wide niche (Spiegel et al, 2007; Ndiritu et al, 2009; Aguilar et al, 2011). By contrast, *Endostelium zonatum* is usually occasional or rare, and it tends to be more common in tropical areas, frequently found on substrates collected in relatively dry habitat and exposed to direct sunlight (Spiegel et al, 2007), and has not been found in boreal climates (Kosheleva et al, 2009; Moore et al, 2000; Spiegel & Stephenson, 2000). *Nematostelium ovatum* is one of the most common species in samples from the lowland tropics but it is also frequent in temperate areas (Spiegel et al, 2007). Finally, *Protostelium pyriforme* is more abundant in the tropics than in temperate areas (Spiegel et al, 2007).

In the CCA (Figure 3) a group of species, most of them with unknown evolutionary affinities (Supplementary Figure S1), showed the tendency to be more abundant in localities with relatively high precipitation and low seasonal changes of temperature and precipitation, and at least some of them were also common in localities with high temperatures. *Microglomus paxillus*, *Echinostelium bisporum*, *Echinosteliopsis oligospora*, *Endostelium zonatum* and *Protostelium arachisporum* are species usually rare in temperate climates and very

rare in higher latitudes, but usually more abundant in tropical localities (Spiegel et al, 2007; Ndiritu et al, 2009). On the other hand, *Protostelium nocturnum* is more abundant in studies from temperate areas (Ndiritu et al, 2009), and also showed affinity for high precipitation in Aguilar et al (2011).

Comparing results from Maxent and CCA, it seems that a group of species has high probability of occurrence in dry areas, usually warm and with moderate to high isothermality. One of them is *Schizoplasmodiopsis vulgaris*, which according to previous data seems to be more common in temperate areas than in tropical or boreal climates (Ndiritu et al, 2009), and it can also be survive in cool, moist habitats (Spiegel et al, 2007). However, *Schizoplasmodiopsis pseudoendospora* is a very abundant species in most localities studied around the world, especially in temperate and tropical areas (Spiegel, 2007), and it also showed preference for warmer temperatures in Aguilar et al (2011). *Protosporangium articulatum*, is a typical inhabitant of bark, so there is little data on it, because this microhabitat is often understudied. However, it has been abundant in some temperate (Ndiritu et al, 2009) and boreal areas (Kosheleva et al, 2009). It appears to be a species that is often associated with arid habitats, and it can occur at higher elevations (>3000 m) than most protostelids (Spiegel et al, 2007). A species that is common worldwide but seems to be more frequent in temperate areas (Spiegel et al, 2007; Ndiritu et al, 2009), *Soliformovum irregulare*, showed preference for higher precipitations and lower temperatures in Aguilar et al (2011). *Cavostelium apophysatum* is found more frequently in tropical areas (Spiegel et al, 2007). However, *Tychosporium acutostipes* does not have a clear latitudinal pattern, but it preferred higher temperatures in Aguilar et al (2011).

Interaction of climate and microhabitat

Microhabitat type also had an important influence on niche segregation, and it has been frequently mentioned as a very important factor in the ecology of these organisms (Olive, 1975; Spiegel, 1986; Spiegel et al, 2004). When studying a locality in detail, species assemblages from each microhabitat frequently differ more than assemblages from the same microhabitat in nearby localities (Moore & Spiegel, 2000b; Spiegel et al, 2004). However, on a continental scale, species composition may vary in each microhabitat at different latitudes (Ndiritu et al, 2009). On the scale of this study, the effect of microhabitats is strong, but it is not known whether their influence is determined by characteristics of the microhabitats themselves – chemical composition, pH, decomposing stage, etc -, or by the assemblage of other interacting organisms that the microhabitats can harbour (Spiegel, 1986). With the methods employed in present research, it is not possible to know in detail the characteristics of each microhabitat type, but they were included in the analyses to get a glimpse of their overall influence on each species, awaiting further characterization in the future.

Results from CCA (Figure 3) show that in the Iberian Peninsula there is a correlation between microhabitat affinity and preference for certain climatic conditions. This correlation is not as strong as when using a more limited set of localities (Aguilar et al, 2011), probably due to the effect of other variables not incorporated in the analyses. With these new results it is possible, however, to visualize the global microhabitat affinity of the species at these latitudes. A

tendency appears that typical ground-litter and bark inhabitants prefer higher temperatures and lower precipitation in winter than the species that appear more frequently in aerial litter, which in turn can tolerate higher precipitation seasonality. As most species considered in this study can survive in at least two different microhabitats, it is also possible that their climatic optima vary in each microhabitat. Clarifying these patterns of interaction between microhabitats and climate is essential for understanding the biogeography of protosteloid amoebae because differential microhabitat selectivity could be a strategy for increasing protostelid ability to tolerate larger climatic and geographic ranges.

Future directions and conclusions

Species' fundamental niche models, and stuSpecies' fundamental niche models, and studies of niche selection can become a very useful tool in the future of protist biogeography. Hypotheses related to the ubiquity of protists' dispersal and its equiprobability in all directions (see Foissner, 2006; Weisse, 2008) deal with the probability of an organism to be transported between suitable habitats, and thus can not be easily falsifiable without an adequate knowledge of the species' ecology. Niche models can be used to generate null hypotheses for an "everything is everywhere" scenario, i. e. they make it possible to identify potential high probability areas and check for the actual presence of the organisms at both sides of a hypothetical barrier.

It has been demonstrated that climate, and other ecological factors interact with diversity to drive macroevolutionary dynamics (Ezard et al, 2011). The comparison of niches of groups of closely related species can also allow us to determine whether niche differentiation has played an important role in their diversification. Niche segregation based on differences in microhabitat and/or tolerated climate ranges may have been a strategy for avoiding high niche overlap and competitive exclusion in co-occurring species, thus permitting coexistence of organisms that compete for the same resources (Pianka, 1974). In this context, a situation that seems to be more common than previously expected in protists and has not been investigated in protostelids yet, is the existence of morphospecies constituted by complexes of cryptic species, which may have distinct ecological preferences and distributions (Amato et al., 2007; Smirnov, 2007; Morard et al., 2009; Douglas et al., 2011). Knowing in more detail the fundamental and realized niches of ecologically similar species can give us new data for analysing all these processes.

In conclusion, the distribution of protosteloid amoebae in the Iberian Peninsula is not random nor spatially autocorrelated, but it is determined by the niche of each organism and the availability of habitats necessary for their survival. Although they share many morphological similarities and have common habitats, each species has its own ecological preferences, determined by their climatic optima and microhabitat colonization capacity. As revealed in this study, the effect of microhabitats is strong and comparable with the effects of climate at the scale of the Iberian Peninsula, but it is not known whether the influence of the microhabitat is due to biotic or abiotic factors, and needs further investigation to clarify the interactions of the microhabitat with external climate. Probably due to the alternating stages in their life cycles, protosteloid amoebae have the ability to resist changes in their environment. As each species can tolerate different types and ranges of change, individual abundances and

species composition of the assemblages vary from locality to locality as the climate changes. These tendencies can be modelled and projected in maps of potential distribution, that constitute hypothesized probabilities of presence given a ubiquitous dispersal, and can be compared with actual presences. This could be a valuable tool in the future for unravelling biogeographic patterns and speciation processes.

Aknowledgements

We wish to thank Alejandro González for extracting values of environmental variables, Fátima Durán for technician work, and Diana Wrigley de Basanta for reviewing the text. This work was supported by the Research Projects CGL2005-00320/BOS and CGL2008-00720/BOS of the Ministry of Science and Innovation of Spain.

Supplementary information is available at ISME Journal's website

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Capítulo 4: Diversidad de los Protostélidos Ibéricos

A continuación se presenta un catálogo comentado de los protostélidos del suroeste de Europa, que recopila todos los registros de protostélidos que se obtuvieron como fruto de los muestreos realizados, incluyendo también las citas correspondientes a las localidades que no pudieron ser incluidas en los análisis realizados en el capítulo anterior.

Aguilar M, Lado C. (2011). Check-list of protostelids from the Southwest of Europe (in prep.)

Resumen: Se ha realizado un estudio de la diversidad de protostélidos en España, Portugal y Francia entre 2005 y 2010. Se recolectaron muestras de tres microhábitat distintos (hojarasca aérea, hojarasca del suelo y corteza de plantas vivas en un total de 97 localidades. Como resultado se registraron 26 especies de las 33 que están descritas hasta la fecha. Se presenta un listado comentado que incluye todos los datos disponibles sobre estos organismos en esta parte del mundo, comentarios sobre la morfología de los esporocarpos, y sobre los estados tróficos. También se incluyen microfotografías de los cuerpos fructíferos de la mayoría de las especies y mapas de distribución.

Check-list of protostelids from the Southwest of Europe

María Aguilar & Carlos Lado

A biodiversity survey for protostelids was carried out in Spain, Portugal and France between 2005 and 2010. Samples were collected from three different microhabitats – aerial litter, ground litter, and bark of living plants – in a total of 97 localities. As result 26 species out of the 33 described to date were recorded. An annotated list that comprises all available data about these organisms from this part of the world, comments on the morphology of the sporocarps and the trophic stages is presented. Microphotographs of the fruiting bodies of most species, and distribution maps are also included.

Introduction

Protostelids, also known as protosteloid amoebae, are a group of heterotrophic unicellular organisms occurring on dead aerial plant parts, bark, leaf litter, and soil from collections made throughout the world, in all continents except Antarctica (Moore & Spiegel, 1995, 2000c; Moore et al, 2000; Spiegel & Stephenson, 2000; Shadwick & Stephenson, 2004; Tesmer et al, 2005; Powers & Stephenson, 2006; Kosheleva et al, 2009; Ndiritu et al, 2009). Their trophic stage varies from uninucleate amoeboid or amoeboflagellate cells to multinucleate reticulate plasmodia, and they form fruiting bodies or sporocarps that are comprised of a single acellular stalk and one to a few spores (Olive, 1975a; Spiegel, 1986; Spiegel et al, 2004). As phagotrophic bacterivores, they probably have an important role in the regulation of the populations of bacteria present in soils and other microhabitats in terrestrial ecosystems (Feest, 1987), where they take part as predators feeding also upon other decomposers such as yeasts, and filamentous fungi (Olive, 1975a; Whitney & Bennett, 1984). These organisms were traditionally classified as occupying a primitive position within the group of slime molds termed Eumycetozoa, that also includes the myxomycetes and the dictyostelids (Olive, 1975a; Spiegel, 1986; Baldauf & Doolittle, 1997), but recent molecular data suggest that protosteloid amoebae are polyphyletic (Shadwick et al, 2009b; Fiore-Donno et al, 2010; Lahr et al, 2011) and they belong to different groups of Amoebozoa, not necessarily directly related to other eumycetozoans.

Several surveys have been carried out in temperate areas (Best & Spiegel, 1984; Moore & Spiegel, 1995, 2000a, 2000b; Shadwick & Stephenson, 2004; Tesmer et al, 2005; Aguilar et al, 2007; Brown & Spiegel, 2008; Shadwick et al, 2009a), tropical regions (Stephenson et al, 1999; Moore & Spiegel, 2000c; Moore & Stephenson, 2003; Powers & Stephenson, 2006; Ndiritu et al, 2009), boreal regions (Spiegel & Stephenson, 2000; Moore et al, 2000; Kosheleva et al, 2009), and aquatic environments (Lindley et al, 2007; Tessmer & Schnittler, 2009). It is remarkable that Europe, one of the territories most extensively studied for the great majority of groups of organisms, has barely been surveyed for protosteloid amoebae. Only one investigation was made in beech

Table 1 – Sampled localities and their characteristics.

	LOCALITY	COORDINATES		ELEV.	SAMPLING DATE	SAMPLES
Loc. 1	Spain, Asturias, Teverga, Vigidel, road TE-1	43.14636ºN	6.14100ºW	630 m	4-X-2005	AS05-1 – AS05-12
Loc. 2	Spain, Asturias, Teverga, Puerto de San Lorenzo, road TE-1	43.14056ºN	6.19333ºW	1310 m	4-X-2005	AS05-13 – AS05-26
Loc. 3	Spain, Asturias, Somiedo, Las Viñas	43.15278ºN	6.26472ºW	740 m	4-X-2005	AS05-27 – AS05-40
Loc. 4	Spain, Asturias, Somiedo, Puerto de Somiedo, road CV-77-3	42.99541ºN	6.20290ºW	1427 m	4-X-2005	AS05-41 – AS05-53
Loc. 5	Spain, Asturias, Somiedo, Saliencia, Endriga	43.10909ºN	6.15511ºW	1300 m	5-X-2005	AS05-54 – AS05-63
Loc. 6	Spain, Asturias, Somiedo, Saliencia, Endriga	43.09000ºN	6.15475ºW	1120 m	5-X-2005	AS05-64 – AS05-69
Loc. 7	Spain, Asturias, Somiedo, Braña Campa d'Abaxu	43.07860ºN	6.13067ºW	1202 m	5-X-2005	AS05-70 – AS05-71
Loc. 8	Spain, Asturias, Somiedo, Saliencia lakes	43.05541ºN	6.09935ºW	1610 m	5-X-2005	AS05-72 – AS05-78
Loc. 9	Spain, Asturias, Somiedo, Alto de la Farragona	43.06147ºN	6.09975ºW	1549 m	5-X-2005	AS05-79 – AS05-84
Loc. 10	Spain, Asturias, Somiedo, La Malva electric power station	43.11275ºN	6.24660ºW	700 m	5-X-2005	AS05-85 – AS05-95
Loc. 11	Spain, Asturias, Somiedo, La Venta Castru, road to Pineda	43.12916ºN	6.26738ºW	534 m	6-X-2005	AS05-96 – AS05-108
Loc. 12	Spain, Asturias, Somiedo, Río Pigüeña	43.14482ºN	6.33294ºW	569 m	6-X-2005	AS05-109 – AS05-121
Loc. 13	Spain, Madrid, Torrelaguna, road N-320, km 337	40.81250ºN	3.58778ºW	815 m	26-X-2006	M06-29 – M06-38
Loc. 14	Spain, Madrid, Casa de Uceda, Pontón de la Oliva	40.88583ºN	3.45528ºW	868 m	26-X-2006	M06-39 – M06-44
Loc. 15	Spain, Guadalajara, Uceda, road to Cubillos de Uceda, km 34	40.82556ºN	3.43528ºW	880 m	26-X-2006	GU06-01 – GU06-06
Loc. 16	Spain, Guadalajara, Usanos, road CM-1008, km 9	40.69889ºN	3.24417ºW	805 m	26-X-2006	GU06-07 – GU06-10
Loc. 17	Spain, Guadalajara, Sacedón, road CM-2000, km 55	40.46000ºN	2.73167ºW	765 m	26-X-2006	GU06-11 – GU06-16
Loc. 18	Spain, Guadalajara, Puebla de Don Francisco, road CM-2000, Jabalera	40.29000ºN	2.76306ºW	670 m	26-X-2006	CU06-01 – CU06-04
Loc. 19	Spain, Guadalajara, Puebla de Don Francisco, road CM-2025, km 4	40.20167ºN	2.73944ºW	800 m	26-X-2006	CU06-05 – CU06-08
Loc. 20	Spain, Madrid, road M-512, km 21	40.41750ºN	4.26389ºW	787 m	19-II-2007	M07-01 – M07-10
Loc. 21	Spain, Madrid, road from Pelayos to San Martín de Valdeiglesias, path to Cerro Valdenoches, km 3	40.34028ºN	4.36167ºW	770 m	19-II-2007	M07-11 – M07-20
Loc. 22	Spain, Ávila, Fresnedilla	40.21056ºN	4.64444ºW	640 m	19-II-2007	AV07-01 – AV07-10
Loc. 23	Spain, Toledo, Real de San Vicente, road CM-5051, km 25	40.17056ºN	4.66111ºW	710 m	19-II-2007	TO07-01 – TO07-10
Loc. 24	Spain, Ávila, Gavilanes	40.27056ºN	4.84500ºW	680 m	19-II-2007	AV07-11 – AV07-20
Loc. 25	Spain, Toledo, Arenas de San Pedro, road from Ávila to Talavera de la Reina	40.11361ºN	5.01194ºW	530 m	19-II-2007	TO07-11 – TO07-20
Loc. 26	Spain, Guadalajara, Aguilar de Anguita, road N-211, km 3	41.03944ºN	2.41861ºW	1162 m	9-VII-2007	GU07-01 – GU07-10
Loc. 27	Spain, Guadalajara, Turmiel, road CM-2107, km 8	41.01222ºN	2.07556ºW	1138 m	9-VII-2007	GU07-11 – GU07-20
Loc. 28	Spain, Teruel, Bañón, Puerto de Bañón	40.83278ºN	1.17500ºW	1227 m	9-VII-2007	TE07-19 – TE07-27
Loc. 29	Spain, Teruel, Mezquita de Jarque, Puerto del Esquinazo, road N-420, km 631	40.71528ºN	0.89667ºW	1425 m	9-VII-2007	TE07-28 – TE07-35
Loc. 30	Spain, Teruel, Castel de Cabra, Puerto de las Traviesas	40.80472ºN	0.67083ºW	1157 m	9-VII-2007	TE07-36 – TE07-42
Loc. 31	Spain, Teruel, Alcañiz, road N-211, km 250	41.09333ºN	0.14139ºW	432 m	9-VII-2007	TE07-43 – TE07-52
Loc. 32	Spain, Zaragoza, Caspe, road Z-221, km 61	41.20750ºN	0.02222ºE	166 m	9-VII-2007	Z07-01 – Z07-10
Loc. 33	Spain, Zaragoza, Cuesta Falcón, road N-211, km 284	41.27333ºN	0.04666ºE	180 m	9-VII-2007	Z07-11 – Z07-20
Loc. 34	Spain, Zaragoza, Los Monegros, Caspe	41.36167ºN	0.10472ºW	335 m	9-VII-2007	Z07-21 – Z07-30
Loc. 35	Spain, Zaragoza, Bujaraloz, Sástago, Montes de la Retuerta, Laguna de la Playa.	41.41139ºN	0.14472ºW	326 m	9-VII-2007	Z07-31 – Z07-38
Loc. 36	Spain, Huesca, Castejón de Monegros, Pallaruelo range	41.62083ºN	0.20750ºW	480 m	9-VII-2007	HU07-01 – HU07-10
Loc. 37	Spain, Huesca, Caldearenas, Puerto de Monrepos, road N-330, km 602	42.35361ºN	0.39194ºW	1312 m	9-VII-2007	HU07-11 – HU07-20
Loc. 38	Spain, Huesca, Sallent de Gállego	42.76222ºN	0.33667ºW	1350 m	9-VII-2007	HU07-22 – HU07-30
Loc. 39	Spain, Huesca, Lanuza, road A-136, km 16	42.75694ºN	0.32083ºW	1308 m	9-VII-2007	HU07-31 – HU07-40
Loc. 40	Spain, Huesca, Canfranc	42.69611ºN	0.52917ºW	1010 m	9-VII-2007	HU07-41 – HU07-48
Loc. 41	Spain, Huesca, Bordas de Lanuza, Tachera (Ansó-Zuriza)	42.85417ºN	0.78861ºW	1287 m	9-VII-2007	HU07-49 – HU07-54
Loc. 42	Spain, Navarra, Isaba, road NA-2000, km 9	42.87556ºN	0.82472ºW	1215 m	10-VII-2007	NA07-01 – NA07-08
Loc. 43	Spain, Navarra, Yesa	42.61722ºN	1.16000ºW	810 m	10-VII-2007	NA07-09 – NA07-16
Loc. 44	Spain, Navarra, Caseda, San Zoilo chapel, road NA-534, km 19	42.50778ºN	1.35083ºW	518 m	10-VII-2007	NA07-17 – NA07-22
Loc. 45	Spain, Navarra, Masadas, road N-121, km 62	42.29861ºN	1.65389ºW	440 m	10-VII-2007	NA07-23 – NA07-34
Loc. 46	Spain, Soria, Olvega, path to Noviercas	41.75139ºN	1.95472ºW	1165 m	10-VII-2007	SO07-01 – SO07-08
Loc. 47	Spain, Soria, Almazán, road CL-101, km 86	41.39417ºN	2.58500ºW	1030 m	10-VII-2007	SO07-09 – SO07-16

Loc. 48	Spain, Soria, Rello, road SO-132, km 38	41.32222ºN	2.74556ºW	1110 m	10-VII-2007	SO07-17 – SO07-24
Loc. 49	Spain, Cuenca, Almonacid del Marquesado, road CM-310, km 5	39.86778ºN	2.78222ºW	763 m	10-V-2007	CU07-01 – CU07-10
Loc. 50	Spain, Cuenca, Motilla del Palancar, Cerros de la Rambla, road N-320, km 68	39.52806ºN	1.89833ºW	833 m	10-V-2007	CU07-11 – CU07-20
Loc. 51	Spain, Cuenca, La Pesquera-Enguidanos, path to Contreras dam	39.59028ºN	1.56222ºW	808 m	10-V-2007	CU07-21 – CU07-30
Loc. 52	Spain, Cuenca, Cepa, road CU-V-5009	39.72639ºN	1.31833ºW	1090 m	10-V-2007	CU07-31 – CU07-38
Loc. 53	Spain, Cuenca, road from Beteta to Masegosa	40.57194ºN	2.06194ºW	1310 m	10-V-2007	CU07-41 – CU07-50
Loc. 54	Spain, Teruel, Albarracín range, Bronchales-Pozondón	40.52250ºN	1.56917ºW	1489 m	10-V-2007	TE07-01 – TE07-08
Loc. 55	Spain, Teruel, road from Nogueras to Albarracín	40.43556ºN	1.58444ºW	1409 m	10-V-2007	TE07-09 – TE07-17
Loc. 56	Spain, Madrid, El Pardo, La Quinta palace	40.50167ºN	3.74000ºW	665 m	24-V-2006	M06-01 – M06-04
Loc. 57	Spain, Madrid, Miraflores de la Sierra	40.80500ºN	3.77750ºW	1085 m	24-V-2006	M06-05 – M06-12
Loc. 58	Spain, Madrid, Miraflores de la Sierra, road to Puerto de la Morcuera	40.83444ºN	3.80056ºW	1450 m	24-V-2006	M06-13 – M06-16
Loc. 59	Spain, Madrid, Puerto de la Morcuera	40.82750ºN	3.83167ºW	1800 m	24-V-2006	M06-17 – M06-20
Loc. 60	Spain, Madrid, El Escorial, road to Abantos	40.59972ºN	4.16056ºW	1215 m	24-V-2006	M06-21 – M06-24
Loc. 61	Spain, Madrid, El Escorial, Abantos	40.60417ºN	4.17361ºW	1540 m	24-V-2006	M06-25 – M06-28
Loc. 62	Spain, Orense, Rubiá, road N-120 to Biobra	42.47278ºN	6.88750ºW	460 m	3-VII-2006	O06-01 – O06-02
Loc. 63	Spain, León, Balboa	42.69500ºN	6.92889ºW	665 m	4-VII-2006	LE06-01 – LE06-02
Loc. 64	Spain, Lugo, Os Ancares, road LU-1401	42.85889ºN	6.88556ºW	1075 m	4-VII-2006	LU06-01 – LU06-02
Loc. 65	Spain, Lugo, Castro, road LU-113	43.15722ºN	7.47528ºW	435 m	5-VII-2006	LU06-03 – LU06-04
Loc. 66	Spain, León, Riaño	42.99028ºN	4.99500ºW	1155 m	6-VII-2006	LE06-03 – LE06-04
Loc. 67	Spain, Palencia, Otero de Guardo	42.90278ºN	4.80083ºW	1270 m	7-VII-2006	PA06-01 – PA06-02
Loc. 68	Spain, Almería, Níjar, San José, road AL-4200, Mirador de las Amatistas	36.82778ºN	2.03917ºW	111 m	1-I-2007	AL07-01 – AL07-02
Loc. 69	Spain, Soria, Navaleno, El Amogable	41.86000ºN	2.94667ºW	1148 m	2-XI-2006	SO06-01
Loc. 70	Spain, Soria, La Muedra, Cuerda del Pozo dam	41.85639ºN	2.80944ºW	1101 m	3-XI-2006	SO06-02
Loc. 71	Spain, Soria, Vinuesa, road from El Rayo to Sotillo del Rincón	41.92667ºN	2.66000ºW	1213 m	3-XI-2006	SO06-03
Loc. 72	Spain, Soria, San Leonardo de Yagüe	41.83611ºN	3.04056ºW	1055 m	4-XI-2006	SO06-04
Loc. 73	Spain, Gerona, Requesens, Roc Colom	42.44917ºN	2.95556ºE	461 m	2-IV-2008	GE08-01 – GE08-10
Loc. 74	Spain, Gerona, Santa Pau, Can Blanc, Fageda d'en Jordà	42.15000ºN	2.52556ºE	762 m	3-IV-2008	GE08-11 – GE08-20
Loc. 75	Spain, Cáceres, Peraleda de la Mata, road EX-118, km 57	39.82415ºN	5.47966ºW	332 m	21-IV-2009	CA09-01 – CA09-10
Loc. 76	Spain, Cáceres, Ibor, road EX-118, km 39	39.68413ºN	5.45146ºW	680 m	21-IV-2009	CA09-11 – CA09-20
Loc. 77	Spain, Cáceres, road EX-118, km 16	39.53715ºN	5.37449ºW	674 m	21-IV-2009	CA09-21 – CA09-30
Loc. 78	Spain, Cáceres, road EX-116 from Guadalupe to Villamayor, km 20	39.25938ºN	5.39103ºW	510 m	21-IV-2009	CA09-31 – CA09-40
Loc. 79	Spain, Badajoz, Quintana de la Serena, road EX-346, km 19	38.84668ºN	5.73782ºW	357 m	21-IV-2009	BA09-01 – BA09-10
Loc. 80	Spain, Badajoz, road EX-103 from Hira de la Serena to Llerena, km 115	38.61056ºN	5.77551ºW	520 m	21-IV-2009	BA09-11 – BA09-21
Loc. 81	Spain, Badajoz, Calera de León, Tentudía monastery, road BA-039, Km 8	38.05285ºN	6.34216ºW	1058 m	22-IV-2009	BA09-22 – BA09-30
Loc. 82	Spain, Huelva, Cañaveral de León, road HU-9108, km 1	38.01902ºN	6.52817ºW	582 m	22-IV-2009	H09-01 – H09-10
Loc. 83	Spain, Huelva, Jabugo	37.92560ºN	6.72140ºW	560 m	22-IV-2009	H09-11 – H09-20
Loc. 84	Portugal, road IP-8 from Beja to Serpa	37.92299ºN	7.50581ºW	220 m	22-IV-2009	PO09-01 – PO09-10
Loc. 85	Portugal, Ourique, road IC-1 to Castro da Cola, Alcaria da Fernão, 2 km away from road N-1	37.57528ºN	8.27965ºW	198 m	22-IV-2009	PO09-11 – PO09-20
Loc. 86	Portugal, São Bartolomeu de Messines, road IC-1, km 719	37.28218ºN	8.28585ºW	112 m	22-IV-2009	PO09-21 – PO09-30
Loc. 87	Spain, Huelva, San Silvestre de Guzmán, road A-499, km 21	37.41976ºN	7.33106ºW	142 m	23-IV-2009	H09-21 – H09-30
Loc. 88	Spain, Huelva, Punta Umbría, El Rápido	37.21355ºN	7.02667ºW	13 m	23-IV-2009	H09-31 – H09-33
Loc. 89	Spain, Huelva, Almonte, road A-486 to El Rocío, km 18	37.21001ºN	6.50505ºW	53 m	23-IV-2009	H09-34 – H09-36
Loc. 90	Spain, Sevilla, El Pedroso, Sierra Norte Natural Park, road A-432, Km 17	37.73501ºN	5.82657ºW	189 m	23-IV-2009	SE09-01 – SE09-10
Loc. 91	Spain, Sevilla, road A-455 from Conastantina to Lora del Río, km 32	37.78101ºN	5.59635ºW	370 m	24-IV-2009	SE09-11 – SE09-13
Loc. 92	Spain, Córdoba, Hornachuelos, Mezquetillas de San Rafael, road COR-530, km 9	37.79406ºN	5.27994ºW	158 m	24-IV-2009	CO09-01 – CO09-10
Loc. 93	Spain, Córdoba, Hornachuelos, road CO-5312, km 5	37.82188ºN	5.25126ºW	154 m	24-IV-2009	CO09-11 – CO09-13
Loc. 94	Spain, Jaén, Quesada, Cazorla range, Prado de las Ubillas fountain	37.84107ºN	2.98643ºW	1454 m	24-IV-2009	J09-01 – J09-03
Loc. 95	Portugal, Loulé, road to Quarteira CM-1297, km 2, close to the station	37.11524ºN	8.05515ºW	104 m	23-IV-2009	PO09-41 – PO09-42
Loc. 96	France, Font Romeu	42.50972ºN	2.046111ºE	1805 m	4-IV-2008	FR08-01 – FR08-10
Loc. 97	France, Mont-Louis	42.50917ºN	2.125556ºE	1541 m	4-IV-2008	FR08-11 – FR08-18

forests in northeastern Germany (Tesmer et al, 2005), one in oak forests of the Ukraine (Glustchenko et al, 2002), and one in taiga forest and steppe of Russia (Kosheleva et al, 2009).

The Iberian Peninsula has previously proved to be an excellent location for other groups of slime moulds, such as Dictyostelids (Romeralo & Lado, 2006) and Myxomycetes (Lado & Pando 1997), and its special features like an accentuated and varied relief, and its varied vegetation and climate produce a high diversity of ecosystems to be colonized by slime molds. It is also characterized by the long-lasting influence of man, constituting a mosaic of successional stages. The study of protostelids in such a wide variety of habitats can help to increase the information about their diversity patterns in areas with temperate climates.

We present here an annotated list of the protostelid species recorded up to date in the southwest of Europe that comprises all available data from this part of the world. Before the beginning of this survey there was no previous information about this group in the study area, and present check-list is the exclusive result of several sampling efforts carried out between 2005 and 2010 by the authors. Some results have been published in Aguilar et al. (2007) and Aguilar et al (2011), being the remining unpublished.

Materials and methods

Samples were collected between 2005 and 2010 in a total of 97 localities (Table 1) in Spain, Portugal and France. All localities were geo-referenced through the use of a portable GPS unit (model Garmin 12, datum WGS 84). Collections of samples were segregated according to microhabitat type - ground litter (layer of twigs, leaves, and other plant debris extending over the soil surface), aerial litter (assemblage of dead but still attached parts of standing plants) or bark. They placed in separate paper bags, air dried and stored with the codes shown in Table 1 in the laboratory of the Real Jardín Botánico.

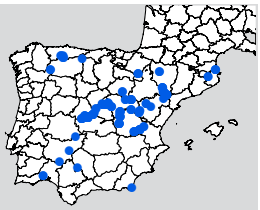
More than 800 primary isolation culture plates were prepared using a modification of the technique described by Olive (1975a), see also Moore & Spiegel (1995), Spiegel et al (2007) and Aguilar et al (2011). The material was cut into small (ca. 1.5-2 cm) pieces with sterile scissors. Thirty-two pieces from each sample were plated out in 8 lines of four pieces forming a circle on a 9 cm Petri dish with a weakly nutrient medium (wMY: 0.002g malt extract, 0.002g yeast extract, 0.75g K_2HPO_4, 15g agar/L of distilled water). The material was moistened with a pipette with sterile water just after been plated out. The plates were incubated at 21ºC and were surveyed for protostelids during the second week of culture.

Species were identified on the basis of fruiting body morphology under the light microscope using both Spiegel et al (2007) and original descriptions. Nomenclature used herein follows Olive (1975a) and Lado (2005-2011). Photomicrographs were taken with a Nikon Eclipse 80i compound microscope using bright field optics and a Nikon Digital Sight DS-5M digital camera head.

Results and discussion

A total of 26 species of protostelids were recorded. Species and comments are listed below.

Cavostelium apophysatum L. S. Olive



OCCURRENCE: **Loc. 1:** ground litter of Compositae, AS05-12. **Loc. 3:** aerial litter of Lamiaceae, AS05-39. **Loc. 6:** bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68. **Loc. 9:** ground litter of *Cytisus* sp., AS05-84. **Loc. 11:** ground litter of *Tilia* sp., AS05-105. **Loc. 13:** ground litter of *Lavandula* sp., M06-32; aerial litter of *Thymus* sp., M06-33; ground litter of *Thymus* sp., M06-34; aerial litter of *Quercus ilex*, M06-35; aerial litter of *Genista scorpius*, M06-37; ground litter of *G. scorpius*, M06-38. **Loc. 14:** ground litter of *Retama sphaerocarpa,* M06-44. **Loc. 15:** ground litter of Leguminosae, GU06-04; ground litter of *Lavandula* sp., GU06-06. **Loc. 16:** ground litter of *Quercus coccifera,* GU06-08; aerial litter of Leguminosae, GU06-09; ground litter of Leguminosae, GU06-10. **Loc. 17:** aerial litter of Gramineae, GU06-11; ground litter of Gramineae, GU06-12; aerial litter of *Rosmarinus officinalis,* GU06-13; ground litter of *R. officinalis,* GU06-14; aerial litter of *Q. coccifera,* GU06-15. **Loc. 18:** ground litter of Gramineae, CU06-02. **Loc. 19:** aerial litter of Gramineae, CU06-05; ground litter of Gramineae, CU06-06; aerial litter of *Thymus* sp., CU06-07; ground litter of *Thymus* sp., CU06-08. **Loc. 20:** aerial litter of *Q. ilex*, M07-05. **Loc. 21:** aerial litter of *Cistus salvifolius*, M07-13; ground litter of *C. salvifolius*, M07-14; ground litter of Gramineae, M07-16; aerial litter of *Lavandula* sp., M07-17; ground litter of *Lavandula* sp., M07-18; bark of *Q. ilex*, M07-19. **Loc. 22:** aerial litter of *Q. ilex*, AV07-03; aerial litter of *Juniperus oxycedrus*, AV07-07; ground litter of *J. oxycedrus*, AV07-08; bark of *Q. ilex*, AV07-09; bark of *J. oxycedrus*, AV07-10. **Loc. 23:** aerial litter of *Q. ilex*, TO07-01; ground litter of *Q. ilex*, TO07-02; ground litter of *R. sphaerocarpa*, TO07-04; aerial litter of *J. oxycedrus*, TO07-05; ground litter of *J. oxycedrus*, TO07-06; aerial litter of *Lavandula* sp., TO07-07; ground litter of *Lavandula* sp., TO07-08; bark of *Q. ilex*, TO07-10. **Loc. 24:** ground litter of *Cistus ladanifer*, AV07-12, aerial litter of *Quercus pyrenaica*, AV07-13, ground litter of *Q. pyrenaica*, AV07-14, aerial litter of *Q. ilex*, AV07-17. **Loc. 25:** aerial litter of *Q. ilex*, TO07-11; aerial litter of thistle, TO07-13; aerial litter of *C. ladanifer*, TO07-15; ground litter of *Lavandula* sp., TO07-18; bark of *Q. ilex*, TO07-20. **Loc. 26:** ground litter of Leguminosae, GU07-06; bark of *J. oxycedrus*, GU07-09. **Loc. 27:** aerial litter of *Juniperus thurifera*, GU07-13; ground litter of *Juniperus thurifera*, GU07-14; aerial litter of *Lavandula* sp., GU07-15; bark of *Juniperus* sp., GU07-16; ground litter of Leguminosae, GU07-18; bark of *Ulmus* sp., GU07-20. **Loc. 28:** ground litter of Lamiaceae, TE07-20; bark of *Q. faginea*, TE07-27. **Loc. 29:** ground litter of *Erinacea anthyllis*, TE07-29. **Loc. 31:** bark of *Olea europaea*, TE07-52. **Loc. 32:** ground litter of *R. officinalis*, Z07-02; bark of *J. phoenicea*, Z07-09. **Loc. 33:** ground litter of Gramineae, Z07-14; bark of *R. officinalis*, Z07-15; bark of *Pinus halepensis*, Z07-16; ground litter of *Pistacia lentiscus*, Z07-20. **Loc. 34:** ground litter of *R. officinalis*, Z07-22; bark of *Juniperus* sp., Z07-23; ground litter of *P. halepensis*, Z07-28. **Loc. 35:** aerial litter of *Lygeum spartum*, Z07-31; aerial litter of *Arthrocnemum* sp., Z07-33; ground litter of *Arthrocnemum* sp., Z07-34; aerial litter of *Suaeda* sp., Z07-36; ground litter of *Salsola* sp., Z07-38. **Loc. 36:** aerial litter of *L. spartum*, HU01-01; ground litter of *L. spartum*, HU01-02; ground litter of Compositae, HU01-06; bark of *R. officinalis*, HU01-09; bark of *J. phoenicea*, HU01-10. **Loc. 37:** aerial litter of *Ulex* sp., HU01-14; bark of *Quercus faginea*, HU01-19. **Loc. 45:** aerial litter of Leguminosae, NA07-23; aerial litter of *Atriplex halimus*, NA07-33; ground litter of *Atriplex halimus*, NA07-34. **Loc. 47:** aerial litter of Lamiaceae, SO07-09; ground litter of Lamiaceae, SO07-10. **Loc. 49:** ground litter of *R. officinalis*, CU07-02; aerial litter of *Cistus albifolius*, CU07-05; ground litter of *Cistus albifolius*, CU07-06; aerial litter of Gramineae, CU07-07; bark of *Q. ilex*, CU07-09; bark of *J. oxycedrus*, CU07-10. **Loc. 50:** ground litter of Compositae, CU07-14; aerial litter of *Q. ilex,* CU07-15; ground litter of *Q. ilex*, CU07-17litter of Leguminosae, M06-26. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01; ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 66:** ground litter of Leguminosae, LE06-04. **Loc. 68:** aerial litter, AL07-01. **Loc. 73:** ground litter of *Erica* sp., GE08-04; aerial litter of *Acer monspessulanum*, GE08-07; bark of *Erica* sp., GE08-09. **Loc. 74:** aerial litter of Rosaceae, GE08-17; bark of *Quercus* sp., GE08-19. **Loc. 78:** aerial litter of Gramineae, CA09-31. **Loc. 80:** aerial litter of *Cistus* sp., BA09-21. **Loc. 81:** ground litter of *Cistus* sp., BA09-22. **Loc. 87:** aerial litter of *Cistus* sp., H09-23; aerial litter of Gramineae, H09-25. **Loc. 92:** aerial litter of *Cistus* sp., CO09-07.

DESCRIPTION: *Sporocarps*- Sporocarps very short stalked, 8-23 µm tall. Stalk 3.2-10.5 µm long, with a distinct, cup-like apophysis. Apophysis from one third to more than one half of the total length of the stalk, usually wider than the base of the stalk, sometimes narrower and the stalk seems to be equally thick for its entire length. Spores rough, colorless, nearly spherical, 4.8-12.6 µm diam, with spines and warts on their surface. Prespore cells circular in outline. (Spiegel et al, 2007).

Trophic stages- It grows well on bacterial cultures including an unidentified bacterium (Florida 20) on wMY agar. The amoebae typically have one single nucleus and one to several contractile vacuoles, and their sub-pseudopodia are filose. When the amoebae are in water, they can develop usually one but quite often two (more rarely three or four) flagella, becoming flagellated cells, mostly 5-13 x 14-32.5 µm. The cysts are spherical to oval or somewhat irregular in shape, 4.3-23 µm in diam (Discover life).

COMMENTS: Though usually is common species in the tropics and relatively uncommon in temperate climates (Spiegel et al, 2007), and it was quite abundant in our study area. It has been also found in Russia (Kosheleva et al 2009).

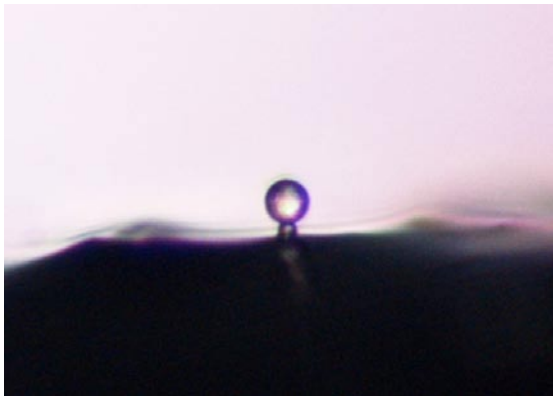
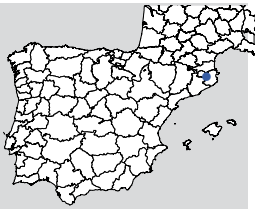


Figure 1 – Fruiting body of *Cavostelium apophysatum.*

***Clastostelium recurvatum* L. S. Olive & Stoian.**



Occurrence: Loc. 74: ground litter of fern, GE08-16.

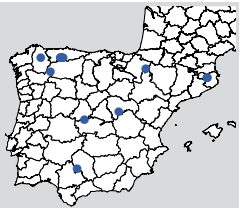
Description: *Sporocarps*- Sporocarps 20-42 µm tall, with two spores at the tip of a bipartite, recurved stalk. Stalk with a short apiculate base and a longer inflated upper portion that bursts to disperse the spores. Spores smooth, hemispherical to subglobose, 7.2-12 µm in diam (Olive & Stoianovitch, 1977a). Prespore cells circular in outline (Spiegel et al, 2007).

Figure 2 – Fruiting body of *Clastostelium recurvatum.*

Trophic stages- It grows well on either hay infusion or lactose-yeast extract agar at pH 6.5-7 in the presence of *Aerobacter aerogenes* or an unidentified pink bacterium isolated from the original substrate. At germination, each spore liberates one or two flagellate cells or a single amoeboid cell, that can be uninucleate or plurinucleate. The cysts are round to ovate or irregular in outline, uninucleate to plurinucleate, 7.2-47 x 7.2-61 µm (Discover life).

Comments: It is a relatively uncommon species that appear to be more frequent in the tropics (Spiegel et al, 2007), we found it only once in our samples.

Echinosteliopsis oligospora Reinhardt & Olive



Occurrence: **Loc. 1:** ground litter of Compositae, AS05-12. **Loc. 2:** aerial litter of *Cytisus* sp., AS05-20. **Loc. 3:** aerial litter of *Cytisus* sp., AS05-31; aerial litter of *Quercus ilex*, AS05-37. **Loc. 11:** ground litter of *Rubus* sp., AS05-97; ground litter of *Campanula* sp., AS05-101; ground litter of Compositae, AS05-103; aerial litter of *Tilia* sp., AS05-104. **Loc. 17:** aerial litter of *R. officinalis,* GU06-13. **Loc. 23:** aerial litter of *J. oxycedrus*, TO07-05. **Loc. 43:** ground litter of Gramineae, NA07-15. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01. **Loc. 65:** aerial litter of *Chamaespartium tridentatum*, LU06-03. **Loc. 74:** aerial litter of *Fagus sylvatica*, GE08-11; aerial litter of fern, GE08-15; aerial litter of Rosaceae, GE08-17. **Loc. 92:** aerial litter of Compositae, CO09-05.

Description: *Sporocarps*- Sporocarps 38-88 µm tall, with a short stalk. Stalk 14-45.5 µ long, broad, straight to slightly curved, tappers from the base to the tip. Spores usually 4-6 in number, surrounded by a transparent, hygroscopic sheath that, in conditions of high humidity, appears as a spherical structure, 17-48 µ in diam, and in dryer conditions deflates, and the sporangium becomes clover-shaped. Prespore cells are circular in outline.

Trophic stages- It can be cultivated on hay infusion agar along with a food organism, like *Flavobacterium* sp., *Escherichia coli*, *Aerobacter aerogenes*, and a mixture of *Phoma conidiigena* and *Flavobacterium*. The spore liberates a single amoeba which is quite broad in movement 28.6-62.1 X 34.5-89.7 µm, and has a distinct hyaloplasmic anterior margin. Posteriorly, fine filose projections are produced. The amoebae are usually uninucleate, but can have up to 4 nuclei. The nuclei have numerous peripheral small nucleoli, differing from that of myxomycetes, which has a single large central nucleolus. No flagellated cells have been observed. The sheath, the spore walls, and the cyst walls give a positive test for cellulose in chloriodide of zinc. The cysts are uninucleate to multinucleate and irregular in outline (Discover life).

Comments: Common worldwide and sometimes locally abundant (Spiegel et al,

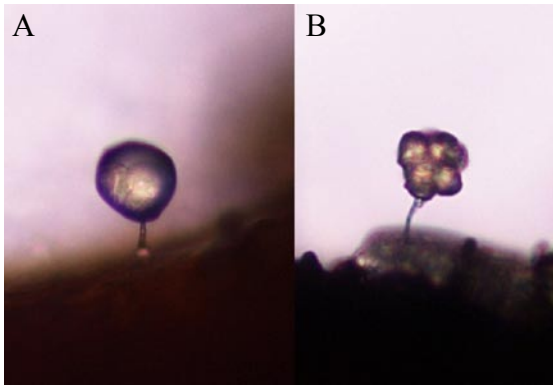
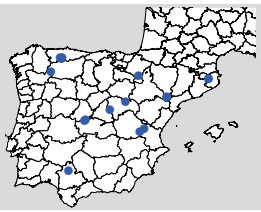


Figure 3 – Fruiting bodies of *Echinosteliopsis oligospora,* A: hydrated, B: dried.

2007), and it was also locally abundant in our cultures. In Europe, this species has been reported previously from Germany (Tesmer et al 2005) and Russia (Kosheleva et al 2009).

Echinostelium bisporum (L. S. Olive & Stoian.) K. D. Whitney & L. S. Olive



Occurrence: **Loc. 2:** aerial litter of *Cytisus* sp., AS05-20. **Loc. 10:** aerial litter of Poaceae, AS05-87. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96. **Loc. 12:** ground litter of *Rubus* sp., AS05-110. **Loc. 16:** aerial litter of Leguminosae, GU06-09. **Loc. 22:** aerial litter of *Q. ilex*, AV07-03. **Loc. 23:** bark of *J. oxycedrus*, TO07-09. **Loc. 26:** ground litter of Leguminosae, GU07-06. **Loc. 32:** bark of *J. phoenicea*, Z07-09. **Loc. 33:** ground litter of Gramineae, Z07-14. **Loc. 45:** ground litter of Leguminosae, NA07-24. **Loc. 51:** bark of *Pinus nigra*, CU07-29. **Loc. 52:** ground litter of Leguminosae, CU07-38. **Loc. 62:** ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 74:** aerial litter of *Fagus sylvatica*, GE08-11; aerial litter of *Castanea sativa*, GE08-13. **Loc. 90:** aerial litter of *Quercus ilex*, SE09-01.

Description: *Sporocarps*- This eumycetozoan has a minute two-spored sporocarp [19-26 µm long] with a seath that can be inflated in high humidity conditions or sticked to the spores in drier stages. One spore [7-10 µm diam] is directly attached to the stalk and the other is at the top. The stalk is short [7-13.5 µm], straight to gently curved and with a pronounced taper from the base to the tip. The prespore cells are circular in outline (Spiegel et al, 2007).
Sporocarps very small, 19-26 µm long, two-spored, with a seath that can be inflated in high humidity conditions or sticked to the spores in drier stages. Stalk short, 7-13.5 µm long, straight to gently curved and with a pronounced taper from the base to the tip. Spore, 7-10 µm diam, one is directly attached to the stalk and the other is at the top. Prespore cells circular in outline (Spiegel et al, 2007).

Trophic stages- They produce amoebae with lobose pseudopodia, flagellated cells, 4-6.5 X 9-19.5 µm, and a plasmodial stage, 32-300 X 54-500 µm. The flagellar aparatus

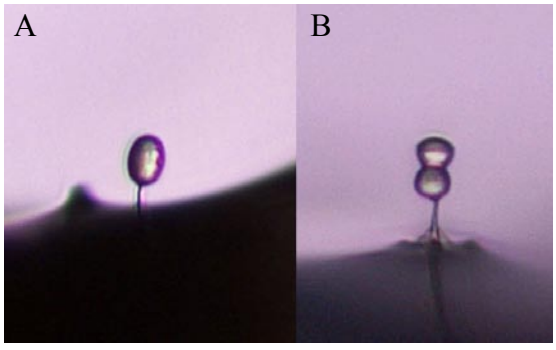


Figure 4 – Fruiting bodies of *Echinostelium bisporum,* A: hydrated, B: dried.

is identical to that of myxomycetes (Spiegel, 1981), having one or two flagella. The cysts are globose or irregular in shape [4.2-22.5 X 4.2-56 µm] (Olive & Stoianovitch, 1966a).

Comments: It is common worldwide but it shows patches of high local abundance (Spiegel et al, 2007), and not very common in our study. It has been reported from Germany (Tesmer et al 2005). This species was first described as a protostelid by Olive & Stoianovitch (1966a) but it is now included in the myxomycetes (Spiegel & Feldman, 1989; Whitney et al, 1982). It is usually studied under the same conditions as protostelids and usually grows intermixed with them.

***Endostelium amerosporum* L. S. Olive**

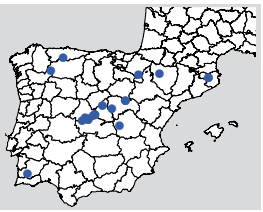
Occurrence: Loc. 28: bark of *Q. faginea*, TE07-27.

Description: Sporocarps- Sporocarps 95-135 µm tall. Stalk 60-87.6 µm, broad, slightly tappered and with a distinct knob like apophysis at the tip. Spores uninucleate, irregular, from elliptical to spherical in shape, 33.6-51.5 µm diam, strongly warted (Olive et al, 1984). Prespore cells are circular in outline Spiegel et al, 2007).

Trophic stages- It grows on lactose-yeast extract and on oak bark pH 6 agar media with *Flavobacterium* sp. added. The amoeba typically contains a nucleus and a contractile vacuole, and it is uninucleate and surrounded by a sheath that contains small particles and frequently bacteria. The cysts, 24-38.4 µm, are typically globose, each usually surrounded by a scabrous sheath (Discover life).

Comments: It is a rare species and has been recorded only a few times (Spiegel et al, 2007), and we found it only in one of our cultures.

Endostelium zonatum (L. S. Olive & Stoian.) W. E. Benn.& L. S. Olive



OCCURRENCE: **Loc. 6:** aerial litter of *Fagus sylvatica*, AS05-64. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07. **Loc. 20:** ground litter of *Lavandula* sp., M07-02; aerial litter of *Q. ilex*, M07-05; ground litter of *Q. ilex*, M07-06. **Loc. 21:** aerial litter of *C. salvifolius*, M07-13. **Loc. 22:** bark of *Q. ilex*, AV07-09. **Loc. 23:** aerial litter of *R. sphaerocarpa*, TO07-03; bark of *Q. ilex*, TO07-10. **Loc. 24:** aerial litter of *Q. pyrenaica*, AV07-13. **Loc. 25:** aerial litter of *Q. ilex*, TO07-11. **Loc. 26:** bark of *J. oxycedrus*, GU07-09; bark of Q. ilex, GU07-10. **Loc. 37:** bark of *Q. faginea*, HU07-19. **Loc. 45:** ground litter of *R. officinalis*, NA07-30. **Loc. 49:** aerial litter of Gramineae, CU07-07. **Loc. 58:** ground litter of Gramineae, M06-16. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01. **Loc. 74:** ground litter of fern, GE08-16; aerial litter of Rosaceae, GE08-17. **Loc. 85:** aerial litter of *Lavandula* sp., PO09-17.

DESCRIPTION: *Sporocarps*- Sporocarps brownish to yellowish, stalked. Stalk 47-120 µm long, beaded, having a chain-like appearance. Spore with variable shape, from somewhat campanulate to elongated or irregular, 14.5-40.5 X 24-46.5 µm, sometimes with warts that appear to be bacteria sticked to the spore surface (Discover life). Prespore cells are slightly ellipsoid to round in outline (Spiegel et al, 2007).

Trophic stages- It grows and sporulates on various bacteria or on combinations of two food organisms such as *Aureobasidium pullulans* and a bacterium, or on two bacteria, according to preference of the particular isolate. Its trophic cells are non-pigmented, most frequently uninucleate but also plurinucleate, usually with a single large contractile vacuole. The amoebae are comparatively large, but they exhibit much variation in cell and nuclear size, and they can develop numerous filose pseudopodia in water. Plurinucleate protoplasts are not uncommon in some cultures, with their number of nuclei ranging from 2 to 16 or more. The cysts are very thin-walled, globose to subglobose or slightly irregular in outline, 22-40 µm diam. (Discover life).

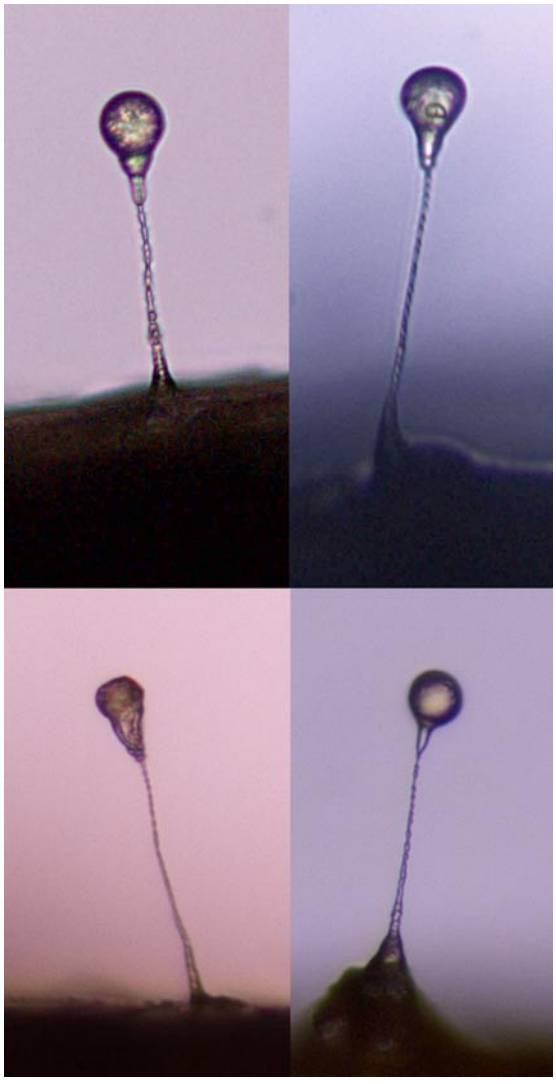
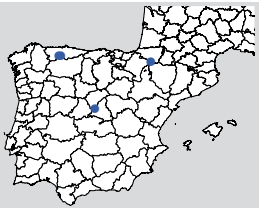


Figure 4 – Fruiting bodies of *Endostelium zonatum*.

COMMENTS: It was originally described

as *Protostelium zonatum* L. S. Olive & Stoian. (Olive & Stoianovitch, 1969). It is quite common worldwide and it is found frequently growing on substrates collected from a relatively dry habitat that is exposed to direct sunlight (Spiegel et al, 2007). It was rare but locally abundant in our cultures.

Microglomus paxillus L. S. Olive & Stoian.



Occurrence: Loc. 2: bark of *Crataegus monogyna*, AS05-26. Loc. 12: bark of *Alnus* sp., AS05-115. Loc. 42: aerial litter of Gramineae, NA07-07. Loc. 61: ground litter of Leguminosae, M06-26.

Description: *Sporocarps*- Sporocarps 22.8-31.2 µm tall, with 2-4 spores. Stalk short, 9.6-16.8 µm long, tapering to form a thin tip. Prespore cells are circular in outline. Spores are compressed against each other forming together an ellipsoidal structure slightly flattened in the upper side, 12-18.5 µm in diam, and can be observed through the sheath. Prespore cells are circular in outline (Olive & Stoianovitch, 1977b).

Trophic stages- It grows and sporulates on soft oak bark agar (at pH 6-6.6) or lactose-yeast extract agar (at pH 6) with a mixture of *Flavobacterium* sp. and another unidentified bacterium (Malaya). The amoebae are uninucleate and they usually have a single contractile vacuole. They have lobose pseudopodia with filose subpseudopodia. No flagellates have been observed. The cysts are spherical to subspherical [10.8-20.4 µm diam] (Discover life).

Comments: It is an uncommon species worldwide (Spiegel et al, 2007), and it was very rarely found during present study.

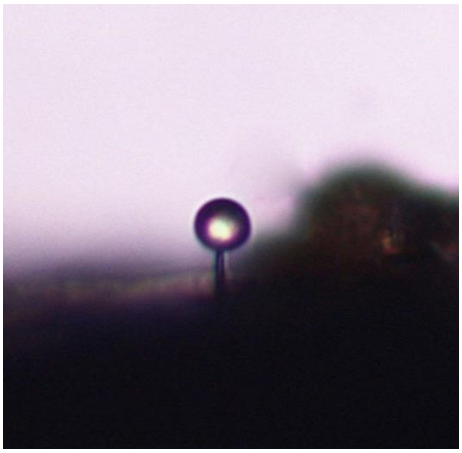
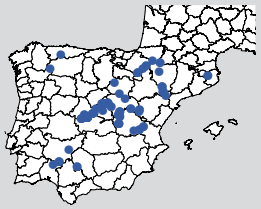


Figure 5 – Fruiting body of *Microglomus paxillus.*

Nematostelium gracile (L. S. Olive & Stoian.) L. S. Olive & Stoian./ *Ceratiomyxella tahitiensis* L. S. Olive & Stoian. complex



OCCURRENCE: **Loc. 3:** aerial litter of Lamiaceae, AS05-39. **Loc. 13:** aerial litter of *Lavandula* sp., M06-31; ground litter of *Thymus* sp., M06-34; aerial litter of *Q. ilex*, M06-35; aerial litter of *G. scorpius*, M06-37; ground litter of *G. scorpius*, M06-38. **Loc. 14:** aerial litter of *R. sphaerocarpa,* M06-43; ground litter of *R. sphaerocarpa,* M06-44. **Loc. 15:** ground litter of Gramineae*,* GU06-02; aerial litter of *Lavandula* sp., GU06-05; ground litter of *Lavandula* sp., GU06-06. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07; ground litter of Leguminosae*,* GU06-10. **Loc. 17:** aerial litter of *R. officinalis,* GU06-13; aerial litter of *Q. coccifera,* GU06-15; ground litter of *Q. coccifera,* GU06-16. **Loc. 18:** aerial litter of Gramineae*,* CU06-01; aerial litter of thistle, CU06-03; ground litter of thistle, CU06-04. **Loc. 19:** aerial litter of Gramineae*,* CU06-05; ground litter of Gramineae*,* CU06-08. **Loc. 20:** ground litter of *Lavandula* sp., M07-02; aerial litter of *Q. ilex*, M07-05. **Loc. 21:** aerial litter of *C. salvifolius*, M07-13; ground litter of *Lavandula* sp., M07-18; bark of *Q. ilex*, M07-19. **Loc. 22:** bark of *Q. ilex*, AV07-09. **Loc. 23:** aerial litter of *Q. ilex*, TO07-01; ground litter of *Q. ilex*, TO07-02; aerial litter of *Lavandula* sp., TO07-07; ground litter of *Lavandula* sp., TO07-08; bark of *J. oxycedrus*, TO07-09. **Loc. 24:** ground litter of *C. ladanifer*, AV07-12; bark of *Q. pyrenaica*, AV07-20. **Loc. 25:** aerial litter of thistle, TO07-13; ground litter of thistle, TO07-14; aerial litter of *Lavandula* sp., TO07-17; ground litter of *Lavandula* sp., TO07-18. **Loc. 26:** ground litter of Leguminosae, GU07-06. **Loc. 32:** aerial litter of Compositae, Z07-03. **Loc. 35:** aerial litter of *Lygeum spartum*, Z07-31; aerial litter of *Arthrocnemum* sp., Z07-33; ground litter of *Arthrocnemum* sp., Z07-34; aerial litter of *Suaeda* sp., Z07-36. **Loc. 36:** ground litter of *Lygeum spartum*, HU07-02; ground litter of Compositae, HU07-06; bark of *J. phoenicea*, HU07-10. **Loc. 37:** ground litter of *Buxus sempervirens*, HU07-12. **Loc. 39:** aerial litter of *Fagus sylvatica*, HU07-34; ground litter of *Rosa* sp., HU07-40. **Loc. 41:** ground litter of fern, HU07-54. **Loc. 43:** ground litter of Leguminosae, NA07-12; ground litter of Gramineae, NA07-15. **Loc. 44:** ground litter of Gramineae, NA07-22. **Loc. 45:** aerial litter of Gramineae, NA07-25; ground litter of Gramineae, NA07-26; ground litter of Cistaceae, NA07-28. **Loc. 48:** ground litter of *Santolina* sp., SO07-20. **Loc.49:** ground litter of *R. officinalis*, CU07-02; aerial litter of *Q. ilex*, CU07-03; aerial litter of *Cistus albifolius*, CU07-05; bark of *Q. ilex*, CU07-09; bark of *J. oxycedrus*, CU07-10. **Loc. 50:** ground litter of Compositae, CU07-14. **Loc. 51:** ground litter of Gramineae, CU07-28; bark of *Pinus nigra*, CU07-29. **Loc. 52:** ground litter of *Q. ilex*, CU07-36; aerial litter of *Q. ilex*, CU07-37. **Loc. 53:** ground litter of *Lavandula* sp., CU07-44; ground litter of Leguminosae, CU07-46. **Loc. 54:** aerial litter of *Cistus* sp., TE07-03; aerial litter of *Cistus* sp., TE07-04. **Loc. 55:** aerial litter of *Q. ilex*, TE07-09. **Loc. 56:** ground litter of *Retama sphaerocarpa*, M06-02. **Loc. 57:** ground litter of Leguminosae, M06-06; ground litter of Leguminosae, M06-12. **Loc. 58:** ground litter of Leguminosae, M06-14. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 62:** ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 72:** ground litter, SO06-04. **Loc. 74:** aerial litter of *Castanea sativa*, GE08-13; bark of Fagus sylvatica, GE08-20. **Loc. 80:** aerial litter of *Cistus* sp., BA09-21. **Loc. 81:** ground litter of *Cistus* sp., BA09-22; aerial litter of Leguminosae, BA09-24; ground litter of Lamiaceae, BA09-28. **Loc. 83:** ground litter of *Cistus* sp., H09-12. **Loc. 92:** aerial litter of Compositae, CO09-05; ground litter of *Cistus* sp., CO09-08.

Two described species share this morphotype, but differ in details of ther life cycles. Studies on this complex are been carried out to clarify whether they are truly distinct (Spiegel et al, 2007).

DESCRIPTION: *Sporocarps*- Stalks 42-240 µm long, stiff, thick and robust, sometimes flexuous and waving in air currents. Distinct knob-like apophysis present. Spores nearly spherical or apically flattened, 11.3-31.3 X 13.8-33.8 µ diam, deciduous. Prespore cells are round from above and hat-shaped from the side (Olive & Stoianovitch, 1971).

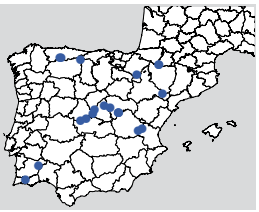
Trophic stages- N. gracile can be cultivated on wMY agar with mixtures of Kitani yeast with Malaya bacterium or of *Cryptococcus larentii* with Malaya bacterium. *C. tahitiensis* grows on malt-yeast extract agar or hay infusion agar pH 6-7.3 with the unidentified bacterium (Malaya) and the unidentified yeast (Kitani) at room temperature or in an incubator at 23º C. They produce a thin, multinucleate, non-reticulate or reticulate plasmodium. The plasmodium divides into irregular multinucleate masses before fruiting. *C. tahitiensis* produces in water anteriorly uniflagellate or occasionally bi-flagellate cells, with or without supernumerary flagella. *N. gracile* does not form flagellates. The cysts are round to irregular in outline (Discover life).

COMMENTS: This species complex is

very frequently found on samples. It is also common temperate regions but it is almost absent at high latitudes and above 2500m (Spiegel et al, 2007). It was very common in our cultures. It has been previously recorded in Germany (Tesmer et al, 2005) and Russia (Kosheleva et al, 2009).

Figure 6 – Fruiting bodies of *Nematosteium gracile / Ceratiomyxella tahitiensis.*

***Nematostelium ovatum* (L. S. Olive & Stoian.) L. S. Olive & Stoian.**



Occurrence: **Loc. 6:** ground litter of *Fagus sylvatica*, AS05-65. **Loc. 10:** ground litter of *Tilia* sp., AS05-94. **Loc. 13:** aerial litter of *Lavandula* sp., M06-31. **Loc. 16:** ground litter of Leguminosae, GU06-10. **Loc. 17:** aerial litter of Gramineae, GU06-11; aerial litter of *R. officinalis,* GU06-13; ground litter of *Q. coccifera,* GU06-16. **Loc. 20:** ground litter of *Lavandula* sp., M07-02. **Loc. 23:** ground litter of *Q. ilex*, TO07-02. **Loc. 25:** aerial litter of *Lavandula* sp., TO07-17. **Loc. 34:** bark of *Juniperus* sp., Z07-23. **Loc. 39:** ground litter of *F. sylvatica*, HU07-32; ground litter of *Quercus* sp., HU07-33. **Loc. 45:** ground litter of Gramineae, NA07-26. **Loc. 51:** bark of *Pinus nigra*, CU07-29. **Loc. 52:** aerial litter of *Q. coccifera*, CU07-31. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 66:** ground litter of Leguminosae, LE06-04. **Loc. 84:** ground litter of *Q. ilex*, PO09-04. **Loc. 86:** ground litter of *Q. suber*, PO09-28.

Description: Sporocarps- Stalks 30-220 µm long, thick and robust with a distinct knob-like apophysis. Spores ovoid to ellipsoid, 10-18.5 X 13-29 µm in diam, deciduous, that have a distinct ring-shaped hilum with a raised edge that fits with the

apophysis of the stalk. Prespore cells round from above and hat-shaped from the side.

Trophic stages- It grows on wMY agar with pre-grown mixtures of Kitani yeast with Malaya bacterium or of *Cryptococcus larentii* with Malaya bacterium. When spores germinate, they produce a thin, multinucleate, branching to reticulate plasmodium, that divides into irregular multinucleate masses before fruiting. The cysts are round to irregular in shape (Discover life).

Comments: This species was originally described as *Schizoplasmodium ovatum* L. S. Olive & Stoian.. It is quite common in temperate areas, and less frequent but also abundant in tropical localities (Spiegel et al, 2007), it was locally common in the Iberian Peninsula. It has been previously recorded in Europe in Germany (Tesmer et al, 2005) and Russia (Kosheleva et al, 2009).

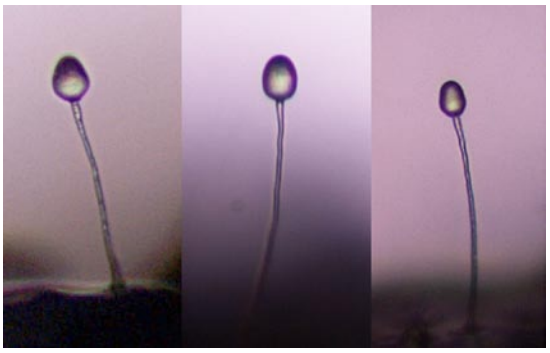
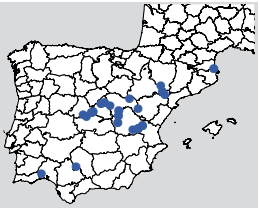


Figure 7 – Fruiting bodies of *Nematosteium ovatum*

***Protosporangium articulatum* L. S. Olive & Stoian.**



Occurrence: **Loc. 13:** aerial litter of *Thymus* sp., M06-33; aerial litter of *Q. ilex*, M06-35; ground litter of *G. scorpius*, M06-38. **Loc. 16:** aerial litter of Leguminosae, GU06-09. **Loc. 17:** aerial litter of *R. officinalis,* GU06-13; aerial litter of *Q. coccifera,* GU06-15. **Loc. 19:** ground litter of Gramineae, CU06-06; aerial litter of *Thymus* sp., CU06-07. **Loc. 20:** aerial litter of *Lavandula* sp., M07-01; aerial litter of *Q. ilex*, M07-05. **Loc. 21:** aerial litter of *Q. ilex*, M07-11; aerial litter of *C. salvifolius*, M07-13; aerial litter of *Lavandula* sp., M07-17. **Loc. 23:** ground litter of *J. oxycedrus*, TO07-06. **Loc. 24:** bark of *Q. pyrenaica*, AV07-20. **Loc. 27:** bark of *Juniperus* sp., GU07-16. **Loc. 32:** bark of *J. phoenicea*, Z07-09. **Loc. 34:** bark of *P. halepensis*, Z07-24; aerial litter of *P. halepensis*, Z07-27. **Loc. 36:** bark of *J. phoenicea*, HU07-10. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; aerial litter of *Cistus albifolius*, CU07-05. **Loc. 50:** ground litter of *Q. ilex*, CU07-16. **Loc. 51:** aerial litter of Leguminosae, CU07-21; aerial litter of Gramineae, CU07-27; ground litter of Gramineae, CU07-28; bark of *Pinus nigra*, CU07-29; bark of *Juniperus* sp., CU07-30. **Loc. 52:** aerial litter of *Q. coccifera*, CU07-31; aerial litter of *Q. ilex*, CU07-37; ground litter of Leguminosae, CU07-38. **Loc. 54:** aerial litter of *Cistus* sp., TE07-03. **Loc. 57:** aerial litter of Leguminosae, M06-05; ground litter of Gramineae, M06-08. **Loc. 73:** ground litter of *Erica* sp., GE08-04; aerial litter of *Acer monspessulanum*, GE08-07. **Loc. 87:** ground litter of *Q. ilex*, H09-21. **Loc. 92:** aerial litter of *Cistus* sp., CO09-07.

Description: *Sporocarps*- Sporocarps 80-185 µm tall, multispored. Stalk proportionally very long and flexuous, with an articulation near the point of attachment to the spore that bends in air currents. They have spherical to ellipsoid structures, formed by hemispherical spores, 5-7.5 x 6.3-10 µm, connected by their flat surfaces. The spores are uninucleate and nondeciduous. Prespore cells are circular in outline (Olive & Stoianovitch, 1972).

Trophic stages- It can be cultivated on

maltose-yeast extract agar at pH 5.1-5.8 in company with Malaya-82 or on oak bark agar. The spores give rise to flagellate cells immediately after germination [11.3-27 x 13.8-35 µm]. The flagella usually occur in pairs (1 long and 1 short). The amoeboid cells have 1 or a few nuclei, but up to 21 nuclei have been observed within a single cell. They do not return to the flagellate stage when placed in water. It has also been observed a vermiform stage, but it is less common than in other members of the genus. The cysts contain 1-5 nuclei, and are globose to oval or occasionally irregular (Discover life).

COMMENTS: Our material shows sporocarps bearing two spores in most cases, but also four-spored sporocarps were observed. They grow frequently on bark and wood. It appears to be a species that is often associated with arid habitats, and it occurs at higher elevations (>3000m) than most protostelids (Spiegel et al, 2007). It was fairly common in our cultures. It was also found in Russia (Kosheleva et al, 2009), France and England (Olive, 1975a).

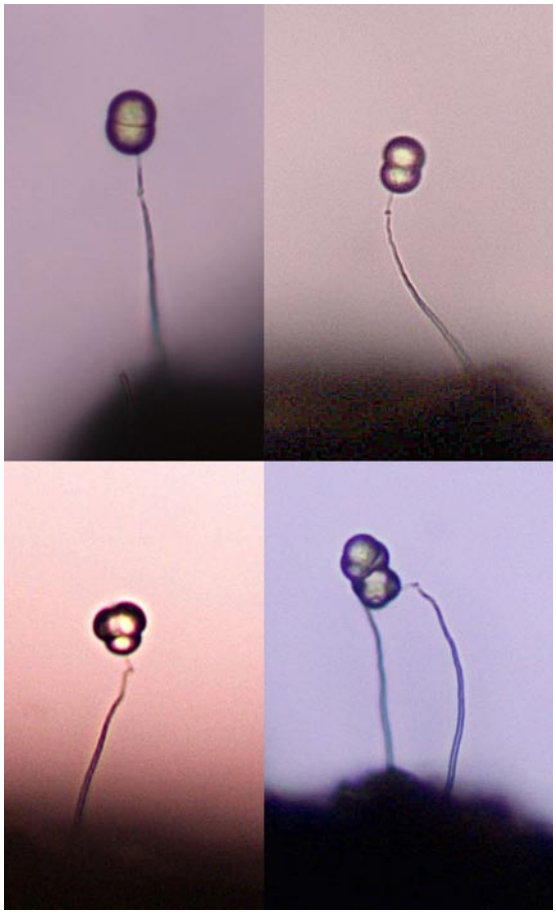


Figure 8 – Fruiting bodies of *Protosporangium articulatum*

Protosporangium bisporum **L. S. Olive & Stoian.**

OCCURRENCE: LOC. 28: aerial litter of Lamiaceae, TE07-19.

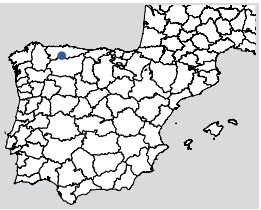
DESCRIPTION: *Sporocarps*- Sporocarps 90-220 µm tall; sporangia globose, 10-13.8 µm in diam. with 1 or 2 spores. Stalks very long, thin and flexuous. Spores hemispherical, 5-7 x 10-13.8 µm, non-deciduous. Prespore cells circular in outline.

Trophic stages- It grows and sporulates

on oak bark agar (at pH 8) with an unidentified bacterium (Malaya) and a moniliaceous fungus (*Goetricum* sp.). The fungus is generally necessary for sporulation, but it may also sporulate in the vicinity of *Penicillium* sp. Single spores give rise to 8 flagellate cells on germination, while spores in pairs produce 4 flagellate cells. Flagellate cells typically have a single anterior flagellum, but occasionally two of equal length are present, and only rarely it is possible to find a short flagellum paired with the longer one. Pseudoflagella (ephemeral filose extensions of the flagellate cell) are commonly seen. Plurinucleate protoplasts that do not develop flagella or become reticulate may be found in cultures several days after spore germination. Protoplasmodia divide by plasmotomy, which tends to limit their size and nuclear number. Under certain conditions, the plurinuceate protoplasts become converted into worm-like shapes. This vermiform phase has an almost segmented appearance, and undulates changing the shape of the swellings constantly. At one or both ends of the protoplast there are knob-like areas with short filose pseudopodia. Cysts round to oval or somewhat irregular, 16.3-33.8 x 22.5-53.8 µm (Discover life).

Comments: This a very uncommon species and it is usually found on bark of living trees, sometimes forming dense patches (Spiegel et al, 2007). It has not been previously reported from Europe.

Protosporangium fragile **L. S. Olive & Stoian.**



Occurrence: Loc. 2: bark of *Crataegus monogyna*, AS05-26.

Description: *Sporocarps*- Sporocarps 65-225 µm tall, that move in air currents. Stalk proportionally long, flexuous and easily fragmented. Spores, 4.3-5.5 x 5.3-7.5, in groups of four, forming structures, 7.5-11 in diam. (Olive & Stoianovitch, 1972). Prespore cells unknown.

Trophic stages- It grows on malt-yeast extract agar with an unidentified bacterium, isolated from hickory bark, as its food source. The species has a restricted pH tolerance in culture and fails to grow if the pH deviates significantly from 5.1.

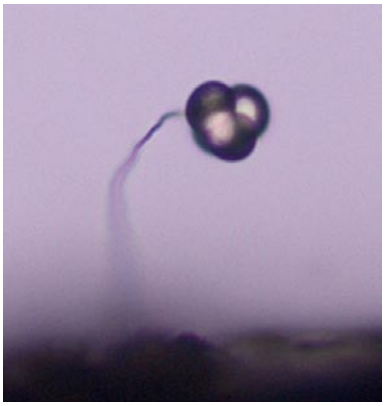
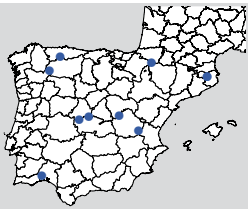


Figure 9 – Fruiting bodies of *Protosporangium fragile*

Each spore germinates giving rise to 2 flagellate cells. The trophic stage consists of uninucleate or plurinucleate ameboid cells, that can produce filose pseudopodia when the cells are placed in water. They can also form a vermiform stage, readily reversible to the flattened ameboid phase. The flagellate cells commonly have either 1 long anterior flagellum, paired with a short reflexed one, or a pair of long flagella. The short flagellum tends to lie against the side of the cell and usually is inconspicuous. Pseudoflagella commonly appear at the apical end of the cell and migrate to the posterior end where they disappear. The nucleus, containing a small central nucleolus, is situated in the more or less hyaloplasmic anterior 1/3 of the cell. The cysts are globose, oval, oblong, or occasionally irregular [10-27 x 13.8-35] (Discover life).

COMMENTS: It is an uncommon species found in most cases growing on bark of living trees or on rotting wood (Spiegel et al, 2007), and it was found only once during this study.

***Protostelium arachisporum* L. S. Olive**



OCCURRENCE: **Loc. 10:** bark of *Pinus sylvestris*, AS05-95. **Loc. 19:** aerial litter of Gramineae, CU06-05. **Loc. 21:** ground litter of *Quercus ilex*, M07-12. **Loc. 25:** bark of *Quercus ilex*, TO07-20. **Loc. 41:** ground litter of fern, HU07-54. **Loc. 51:** aerial litter of Leguminosae, CU07-21. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01. **Loc. 74:** ground litter of Castanea sativa, GE08-14; bark of *Quercus* sp., GE08-19. **Loc. 87:** bark of *Q. ilex*, H09-29.

DESCRIPTION: *Sporocarps*- Stalk, 19.5-62.5 µm long, relatively long, narrow, with a small knob-like apophysis. Spores very variable in shape, from almost spherical or ovate to elongate with one or more constrictions resembling the pod of a peanut, 8.8-9.3 x 20-46 µm. Prespore cells slightly ellipsoid to round in outline (Olive, 1962).

Trophic stages- It grows on LY or wMY agar, with *Flavobacterium* sp.. The fan-shaped amoebae have filose subpseudopodia and a single distinct nucleus. They present a contractile vacuole, and a scalloped, hyaline anterior border when migrating across the agar surface. The microcysts are spherical and thin-walled, 10-2.8 µm diam. (Discover life).

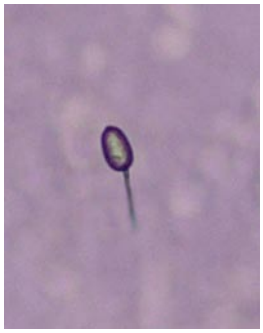
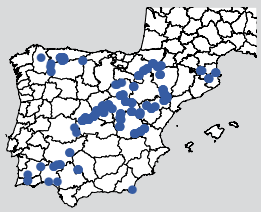


Figure 10 – Fruiting body of *Protostelium arachisporum.*

COMMENTS: This fairly common species

is more abundant in the tropics than in temperate areas. It probably represents a species complex and is unlikely to be a member of the eumycetozoans (Spiegel et al, 2007). It was quite uncommon in our cultures, and it has been previously recorded in Germany (Tesmer et al, 2005) and Russia (Kosheleva et al, 2009).

***Protostelium mycophagum* L. S. Olive & Stoian. complex / *Planoprotostelium aurantium* L.S.Olive & Stoian.**



OCCURRENCE: **Loc. 1:** aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of Compositae, AS05-11; ground litter of Compositae, AS05-12. **Loc. 2:** aerial litter of *Cytisus* sp., AS05-20; ground litter of thistle, AS05-23; ground litter of *Crataegus monogyna*, AS05-25. **Loc. 3:** ground litter of *Cytisus* sp., AS05-32; aerial litter of *Hedera helix*, AS05-35; aerial litter of Lamiaceae, AS05-39. **Loc. 4:** aerial litter of *Erica* sp., AS05-48; aerial litter of *Mentha* sp., AS05-52; ground litter of *Mentha* sp., AS05-53. **Loc. 5:** aerial litter of *Corylus avellana*, AS05-62. **Loc. 6:** aerial litter of *Fagus sylvatica*, AS05-64. **Loc. 9:** aerial litter of Lamiaceae, AS05-81; aerial litter of Lamiaceae, AS05-82; aerial litter of *Cytisus* sp., AS05-83. **Loc. 10:** aerial litter of Poaceae, AS05-87; aerial litter of *Aesculus hippocastanum*, AS05-88; bark of *Pinus sylvestris*, AS05-95. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; aerial litter of Compositae, AS05-102; ground litter of Compositae, AS05-103; aerial litter of *Tilia* sp., AS05-104. **Loc. 12:** aerial litter of *Rubus* sp., AS05-109; ground litter of *Rubus* sp., AS05-110; aerial litter Lamiaceae, AS05-111; aerial litter of *Alnus* sp., AS05-113; ground litter of Cyperaceae, AS05-117; ground litter of *Rumex* sp., AS05-118; ground litter of *Equisetum* sp., AS-121. **Loc. 13:** aerial litter of Gramineae, M06-29; ground litter of Gramineae, M06-30; aerial litter of *Lavandula* sp., M06-31; ground litter of *Lavandula* sp., M06-32; aerial litter of *Thymus* sp., M06-33; ground litter of *Thymus* sp., M06-34; aerial litter of *Q. ilex*, M06-35; ground litter of *G. scorpius*, M06-38. **Loc. 14:** aerial litter of *C. ladanifer*, M06-39; ground litter of *C. ladanifer*, M06-40; aerial litter of Gramineae, M06-41; ground litter of Gramineae, M06-42; aerial litter of *R. sphaerocarpa,* M06-43. **Loc. 15:** aerial litter of Gramineae, GU06-01; ground litter of Gramineae, GU06-02; aerial litter of Leguminosae, GU06-03; ground litter of Leguminosae, GU06-04; aerial litter of *Lavandula* sp., GU06-05; ground litter of *Lavandula* sp., GU06-06. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07; ground litter of *Q. coccifera,* GU06-08; aerial litter of Leguminosae, GU06-09. **Loc. 17:** aerial litter of Gramineae, GU06-11; ground litter of Gramineae, GU06-12; aerial litter of *R. officinalis,* GU06-13; ground litter of *R. officinalis,* GU06-14; aerial litter of *Q. coccifera,* GU06-15. **Loc. 18:** aerial litter of Gramineae, CU06-01; ground litter of Gramineae, CU06-02; aerial litter of thistle, CU06-03; ground litter of thistle CU06-04. **Loc. 19:** aerial litter of Gramineae, CU06-05; ground litter of Gramineae, CU06-06; aerial litter of *Thymus* sp., CU06-07; ground litter of *Thymus* sp., CU06-08. **Loc. 20:** aerial litter of *Lavandula* sp., M07-01; ground litter of *Lavandula* sp., M07-02; aerial litter of *R. sphaerocarpa*, M07-03; ground litter of *R. sphaerocarpa*, M07-04; aerial litter of *Q. ilex*, M07-05; aerial litter of Gramineae, M07-07; ground litter of Gramineae, M07-08. **Loc. 21:** aerial litter of *Q. ilex*, M07-11; aerial litter of *C. salvifolius*, M07-13; ground litter of *C. salvifolius*, M07-14; aerial litter of Gramineae, M07-15; aerial litter of *Lavandula* sp., M07-17; ground litter of *Lavandula* sp., M07-18. **Loc. 22:** aerial litter of Leguminosae, AV07-01; ground litter of Leguminosae, AV07-02; aerial litter of *Q. ilex*, AV07-03; aerial litter of Gramineae, AV07-05. **Loc. 23:** aerial litter of *Q. ilex*, TO07-01; aerial litter of *R. sphaerocarpa*, TO07-03; ground litter of *R. sphaerocarpa*, TO07-04; aerial litter of *J. oxycedrus*, TO07-05; ground litter of *J. oxycedrus*, TO07-06; aerial litter of *Lavandula* sp., TO07-07; bark of *Q. ilex*, TO07-10. **Loc. 24:** aerial litter of *C. ladanifer*, AV07-11; aerial litter of *Q. pyrenaica*, AV07-13; ground litter of *Quercus pyrenaica*, AV07-14; aerial litter of *Rubus* sp., AV07-15; ground litter of *Rubus* sp., AV07-16. **Loc. 25:** aerial litter of *Q. ilex*, TO07-11; aerial litter of thistle, TO07-13; ground litter of thistle, TO07-14; ground litter of *C. ladanifer*, TO07-16; aerial litter of *Lavandula* sp., TO07-17; bark of *Q. ilex*, TO07-20. **Loc. 26:** aerial litter of Gramineae, GU07-01; ground litter of Gramineae, GU07-02; aerial litter of *Santolina* sp., GU07-03; ground litter of *Santolina* sp., GU07-04; aerial litter of Leguminosae, GU07-05; aerial litter of *Thymus* sp., GU07-07; bark of *J. oxycedrus*, GU07-09. **Loc. 27:** aerial litter of *Juniperus thurifera*, GU07-13; aerial litter of *Lavandula* sp., GU07-15; bark of *Juniperus* sp., GU07-16; aerial litter of Leguminosae, GU07-17; ground litter of Leguminosae, GU07-18; bark of *Ulmus* sp., GU07-20. **Loc. 28:** aerial litter of Lamiaceae, TE07-19; ground litter of Lamiaceae, TE07-20; aerial litter of *Q. faginea*, TE07-25; bark of *Q. faginea*, TE07-27. **Loc. 29:** aerial litter of *Erinacea anthyllis*, TE07-28; aerial litter of Lamiaceae, TE07-30; ground litter of Gramineae, TE07-33; aerial litter of Brassicaceae, TE07-34. **Loc. 30:** aerial litter of Compositae, TE07-38; ground litter of Leguminosae, TE07-41. **Loc. 31:** aerial litter of Lamiaceae, TE07-45; aerial litter of Cistaceae, TE07-47; ground litter of Cistaceae, TE07-48; aerial litter of Gramineae, TE07-49. **Loc. 32:** aerial litter of *R. officinalis*, Z07-01; aerial litter of Compositae, Z07-03; aerial litter of Gramineae, Z07-05. **Loc. 33:** ground litter of Gramineae, Z07-14; bark of *Pinus halepensis*, Z07-16; aerial litter of *Pistacia lentiscus*, Z07-19. **Loc. 34:** bark of *Juniperus* sp., Z07-23; aerial litter of Gramineae, Z07-25; aerial litter of *P. halepensis*, Z07-27. **Loc. 35:** aerial litter of *Lygeum spartum*, Z07-31; aerial litter of *Arthrocnemum* sp., Z07-33; aerial litter of *Suaeda* sp., Z07-35; aerial litter of *Salsola* sp., Z07-37. **Loc. 36:** aerial litter of *Lygeum spartum*, HU07-01; ground litter of *Lygeum spartum*, HU07-02; aerial litter of Lamiaceae, HU07-03; aerial litter of Compositae, HU07-05; ground litter of Compositae, HU07-06; bark of *J. phoenicea*, HU07-10. **Loc. 37:** aerial litter of *Buxus sempervirens*, HU07-11; aerial litter of *Ulex* sp., HU07-13; aerial litter of Gramineae, HU07-15; ground litter of Gramineae, HU07-16; aerial litter of *Arctostaphyllos uva-ursi*, HU07-18; bark of *Q. faginea*, HU07-19. **Loc. 38:** bark of *Salix* sp., HU07-23; aerial litter of *Geum* sp., HU07-27; ground litter of *Geum* sp., HU07-28. **Loc. 39:** aerial litter of *Populus tremula*, HU07-35; aerial litter of *Rosa* sp., HU07-39. **Loc. 40:** aerial litter of Gramineae, HU07-41; ground litter of *Buxus sempervirens*, HU07-44; aerial litter of Leguminosae, HU07-47. **Loc. 41:** ground litter of *F. sylvatica*, HU07-52; aerial litter of fern, HU07-53; ground litter of fern, HU07-54. **Loc. 42:** aerial litter of *F. sylvatica*, NA07-03; aerial litter of *P. sylvestris*, NA07-05; aerial litter of Gramineae, NA07-07; ground litter of Gramineae, NA07-08. **Loc. 43:** aerial litter of *Q. humilis*, NA07-09; ground litter of *Q. humilis*, NA07-10; aerial litter of Leguminosae, NA07-11; aerial litter of Gramineae, NA07-13; ground litter of Gramineae, NA07-15. **Loc. 44:** aerial litter of *B. sempervirens*, NA07-17; aerial litter of *Q. coccifera*, NA07-19; ground litter of *Q.*

coccifera, NA07-20; aerial litter of Gramineae, NA07-21. **Loc. 45:** aerial litter of Leguminosae, NA07-23; aerial litter of Gramineae, NA07-25; ground litter of Gramineae, NA07-26; aerial litter of Cistaceae, NA07-27; aerial litter of *R. officinalis*, NA07-29; ground litter of Compositae, NA07-32; aerial litter of *Atriplex halimus*, NA07-33. **Loc. 46:** aerial litter of *Q. pyrenaica*, SO07-01; aerial litter of *J. communis*, SO07-03; ground litter of Gramineae, SO07-06; aerial litter of *Q. ilex*, SO07-07; ground litter of Q. ilex, SO07-08. **Loc. 47:** aerial litter of Lamiaceae, SO07-09; ground litter of Gramineae, SO07-14; ground litter of Leguminosae, SO07-16. **Loc. 48:** ground litter of Lamiaceae, SO07-18; aerial litter of *Santolina* sp., SO07-19; ground litter of *Santolina* sp., SO07-20; aerial litter of Gramineae, SO07-22. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; aerial litter of *Cistus albifolius*, CU07-05; ground litter of *Cistus albifolius*, CU07-06; aerial litter of Gramineae, CU07-07; ground litter of Gramineae, CU07-08; bark of *J. oxycedrus*, CU07-10. **Loc. 50:** aerial litter of *Thymus* sp., CU07-11; aerial litter of Compositae, CU07-13; ground litter of Compositae, CU07-14; aerial litter of *Q. ilex*, CU07-15; ground litter of *Q. ilex*, CU07-16; ground litter of Gramineae, CU07-18; bark of *Pinus* sp., CU07-20. **Loc. 51:** ground litter of Leguminosae, CU07-22; aerial litter of *Thymus* sp., CU07-23; ground litter of *Thymus* sp., CU07-24; aerial litter of *R. officinalis*, CU07-25; ground litter of *R. officinalis*, CU07-26; aerial litter of Gramineae, CU07-27; ground litter of Gramineae, CU07-28; bark of *Pinus nigra*, CU07-29; bark of *Juniperus* sp., CU07-30. **Loc. 52:** aerial litter of *Q. coccifera*, CU07-31; aerial litter of *Q. ilex*, CU07-35; ground litter of *Q. ilex*, CU07-36; aerial litter of *Q. ilex*, CU07-37; ground litter of Leguminosae, CU07-38. **Loc. 53:** aerial litter of *Thymus* sp., CU07-41; ground litter of *Thymus* sp., CU07-42; aerial litter of *Lavandula* sp., CU07-43; ground litter of *Lavandula* sp., CU07-44; aerial litter of Leguminosae, CU07-45; ground litter of Gramineae, CU07-48. **Loc. 54:** aerial litter of *J. communis*, TE07-01; aerial litter of *Cistus* sp., TE07-04; aerial litter of Rosaceae, TE07-06. **Loc. 55:** aerial litter of *Q. ilex*, TE07-09; ground litter of *Q. ilex,* TE07-10; aerial litter of *Cistus* sp., TE07-11; bark of *Q. ilex*, TE07-17. **Loc. 56:** aerial litter of *Retama sphaerocarpa*, M06-01; ground litter of *Retama sphaerocarpa*, M06-02; ground litter of Gramineae, M06-04. **Loc. 57:** aerial litter of Leguminosae, M06-05; aerial litter of Gramineae, M06-07; ground litter of Gramineae, M06-08; aerial litter of Leguminosae, M06-09; aerial litter of Leguminosae, M06-11; ground litter of Leguminosae, M06-12. **Loc. 58:** aerial litter of Leguminosae, M06-13; ground litter of Leguminosae, M06-14; ground litter of Gramineae, M06-16. **Loc. 59:** ground litter of Leguminosae, M06-18. **Loc. 60:** aerial litter of Leguminosae, M06-21; ground litter of Leguminosae, M06-22; ground litter of Gramineae, M06-24. **Loc. 61:** aerial litter of Leguminosae, M06-25; ground litter of Leguminosae, M06-26. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01; ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 63:** aerial litter of Gramineae, LE06-01. **Loc. 64:** aerial litter of Leguminosae, LU06-01. **Loc. 65:** aerial litter of *Chamaespartium tridentatum*, LU06-03. **Loc. 66:** aerial litter of Leguminosae, LE06-03; ground litter of Leguminosae, LE06-04. **Loc.68:** ground litter, AL07-02. **Loc. 69:** ground litter, SO06-01. **Loc. 71:** ground litter, SO06-03. **Loc. 72:** ground litter, SO06-04. **Loc. 73:** aerial litter of *Erica* sp., GE08-03; ground litter of *Erica* sp., GE08-04; aerial litter of *Quercus suber*, GE08-05; ground litter of *Quercus suber*, GE08-06; aerial litter of *Acer monspessulanum*, GE08-07. **Loc. 74:** aerial litter of *Fagus sylvatica*, GE08-11; ground litter of *Fagus sylvatica*, GE08-12; aerial litter of *Castanea sativa*, GE08-13; aerial litter of Rosaceae, GE08-17; ground litter of Rosaceae, GE08-18. **Loc. 75:** bark of *Q. ilex*, CA09-09. **Loc. 77:** ground litter of Gramineae, CA09-24. **Loc. 80:** aerial litter of Gramineae, BA09-11; aerial litter of Leguminosae, BA09-15; aerial litter of *Cistus* sp., BA09-21. **Loc. 81:** aerial litter of Leguminosae, BA09-24; aerial litter of Gramineae, BA09-25; ground litter of Gramineae, BA09-26; aerial litter of Lamiaceae, BA09-27. **Loc. 82:** aerial litter of Lamiaceae, H09-07. **Loc. 83:** ground litter of *Cistus* sp., H09-12; aerial litter of *Q. suber*, H09-17. **Loc. 84:** aerial litter of Gramineae, PO09-01; ground litter of Gramineae, PO09-02. **Loc. 85:** aerial litter of *Cistus* sp., PO09-11. **Loc. 86:** aerial litter of *Cistus* sp., PO09-21; aerial litter of *Q. ilex*, PO09-27. **Loc. 88:** bark, H09-33. **Loc. 89:** ground litter, H09-35. **Loc. 92:** aerial litter of Gramineae, CO09-01; ground litter of Gramineae, CO09-02; aerial litter of *Q. ilex*, CO09-03; aerial litter of Compositae, CO09-05; ground litter of Compositae, CO09-06; aerial litter of *Cistus* sp., CO09-07. **Loc. 96:** ground litter of thistle, FR08-02; aerial litter of Gramineae, FR08-03; ground litter of gramineae, FR08-04; ground litter of Compositae, FR08-06; aerial litter of Rosaceae, FR08-07; ground litter of Compositae, FR08-10. **Loc. 97:** aerial litter of *Betula* sp., FR08-17.

DESCRIPTION: *Sporocarps*- Most individuals have sporocarps that move easily in air currents, others have piliform sections in their stalks that float and curl continuously, even in the absence of evident air currents, while others present stiffer stalks. Stalks less than 70 µm long, tapered at maturity, flexuous, and flexible, often presenting a small apophysis at their tips. Spores spherical to slightly obpyriform , 8.8-13.8 µm in diam., smooth (Olive & Stoianovitch, 1969). Prespore cells elliptical when viewed from above.

Trophic stages- The amoebae can feed upon bacteria (*Flavobacterium* sp. and *Aerobacter aerogenes*) as well as fungi (Olive & Stoianovitch, 1969). They are uninucleate, bigger than those of *P. nocturnum* and the nucleus containins a prominent nucleolus in interphase. Amoebae contain one to three prominent contractile vacuoles and numerous pink to orange lipid droplets. Migrating amoebae produce broad, lamellate pseudopodia, with some blunt to acutely pointed subseudopodia extending from them. More elongated, pointed subpseudopodia (filose pseudopodia, sensu Olive, 1975a) are found under wetter conditions. The shape of the amoebae varies from irregularly circular to elongate to occasionally flabellate. Amoebae may move by the gently eruptive production of pseudopodia that subsequently appear to pull the cell along or they may glide along the substratum by some as yet unknown mechanism. Gliding is more rapid than pseudopodial crawling. They have distinct three-dimensional relief when viewed on the surface of a culture plate (Spiegel et al, 1994).

COMMENTS: This species is very variable in its morphology, both in size and deciduousness of spores, and probably

constitutes a species complex (Spiegel et al, 2007). This is one of the most frequently encountered species worldwide (Spiegel et al, 2007). In Europe, this species has been reported from Holland (Olive, 1962, 1967), Sweden (Olive, 1962, 1967), Greece (Olive, 1967), Germany (Tesmer et al, 2005) and Russia (Kosheleva et al, 2009).

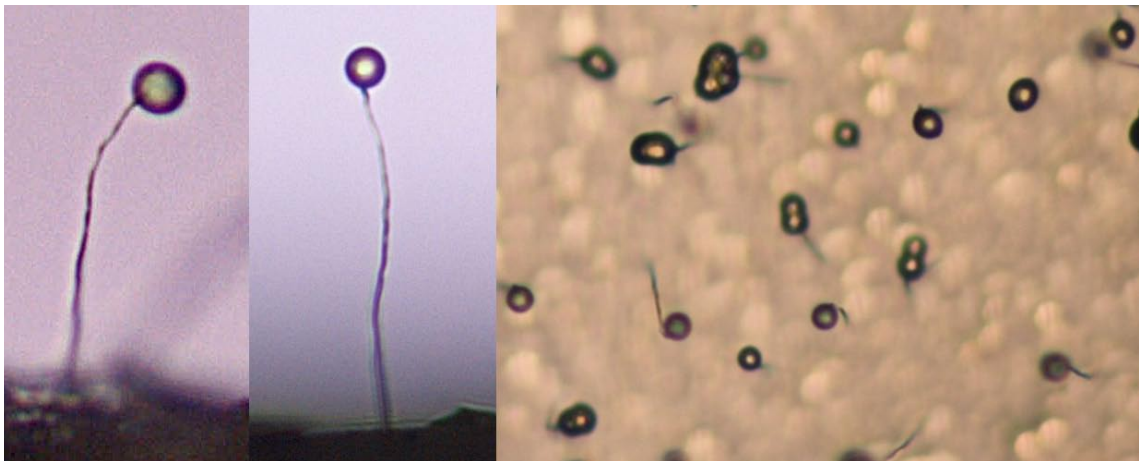
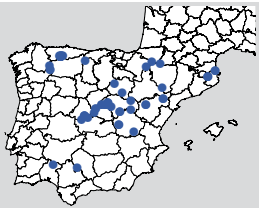


Figure 11 – Fruiting bodies of *Protostelium mycophagum*

***Protostelium nocturnum* Spiegel**



OCCURRENCE: **Loc. 1:** aerial litter of *Pteridium aquilinum*, AS05-5. **Loc. 2:** ground litter of thistle, AS05-23. **Loc. 3:** ground litter of *Cytisus* sp., AS05-32; ground litter of *Hedera helix*, AS05-36. **Loc. 10:** ground litter of *Tilia* sp., AS05-94. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; aerial litter of Compositae, AS05-102; ground litter of Compositae, AS05-103; aerial litter of *Tilia* sp., AS05-104. **Loc. 12:** aerial litter of Lamiaceae, AS05-111. **Loc. 13:** ground litter of Gramineae, M06-30; aerial litter of *Lavandula* sp., M06-31; aerial litter of *Thymus* sp., M06-33; ground litter of *G. scorpius*, M06-38. **Loc. 14:** aerial litter of C. ladanifer, M06-39; aerial litter of Gramineae, M06-41; aerial litter of *R. sphaerocarpa,* M06-43. **Loc. 15:** aerial litter of *Lavandula* sp., GU06-05. **Loc. 16:** aerial litter of Leguminosae, GU06-09. **Loc. 17:** aerial litter of *R. officinalis,* GU06-13; aerial litter of *Q. coccifera,* GU06-15. **Loc. 20:** aerial litter of *Q. ilex*, M07-05. **Loc. 22:** aerial litter of *Q. ilex*, AV07-03. **Loc. 24:** aerial litter of *Q. pyrenaica*, AV07-13; ground litter of *Quercus pyrenaica*, AV07-14; ground litter of *Rubus* sp., AV07-16. **Loc. 25:** aerial litter of *Q. ilex*, TO07-11. **Loc. 27:** aerial litter of *Lavandula* sp., GU07-15. **Loc. 28:** aerial litter of Lamiaceae, TE07-19; bark of *Q. faginea*, TE07-27. **Loc. 31:** ground litter of Cistaceae, TE07-48; aerial litter of Gramineae, TE07-49. **Loc. 36:** aerial litter of *Lygeum spartum*, HU07-01. **Loc. 39:** aerial litter of *Fagus sylvatica*, HU07-34. **Loc. 42:** aerial litter of *Rosa* sp., NA07-01. **Loc. 43:** aerial litter of *Q. coccifera*, NA07-16. **Loc. 47:** aerial litter of Lamiaceae, SO07-09. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; aerial litter of *Cistus albifolius*, CU07-05. **Loc. 50:** aerial litter of *Q. ilex*, CU07-15. **Loc. 53:** aerial litter of *Lavandula* sp., CU07-43. **Loc. 58:** ground litter of Leguminosae, M06-14. **Loc. 59:** ground litter of Leguminosae, M06-18. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 61:** ground litter of Leguminosae, M06-26. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01; ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 63:** aerial litter of Gramineae, LE06-01. **Loc. 67:** ground litter of Leguminosae, PA06-02. **Loc. 72:** ground litter, SO06-04. **Loc. 73:** aerial litter of *Erica* sp., GE08-03; ground litter of *Erica* sp., GE08-04. **Loc. 74:** aerial litter of *Fagus sylvatica*, GE08-11; aerial litter of *Castanea sativa*, GE08-13; aerial litter of Rosaceae, GE08-17. **Loc. 83:** aerial litter of Gramineae, H09-15. **Loc. 92:** ground litter of *Cistus* sp., CO09-08.

Description: *Sporocarps*- Sporocarps similar in shape to those of *P. mycophaga*, but smaller in size. Stalk (15.6-)18-26(31.2) µm long. Spores nearly spherical, (6.5-)7.5-10.4 µm in diam., smooth, soon actively released with the disappearance of the stalk (Spiegel, 1984). Prespore cells elliptical.

Trophic stages- It grows on wMY agar or hay infusion agar with *Xanthomonas fragariae* (Fla-20 isolate of Olive) or *Rhodotorula mucilaginosa*, and on CM+ agar with *Rhodotorula*. It grows well but fruits poorly on CM+ with X. fragariae. The amoebae are small, uninucleate, and orange-pigmented, and they have a nucleus with a single, central nucleolus, and one or more contractile vacuoles and many food vacuoles. Orange pigmented lipid droplets are also present. They are relatively smooth in outline on dry agar, but acutely pointed pseudopodia and lamellopodia become increasingly prominent as the medium becomes more liquid. The microcysts are spherical or ellipsoidal (Discover life).

Comments: This species fruit most heavily after sunset until early morning. It is relatively common worldwide (Spiegel et al, 2007) and also common in the Iberian peninsula. This species has been cited in Europe for Germany (Tesmer et al, 2005).

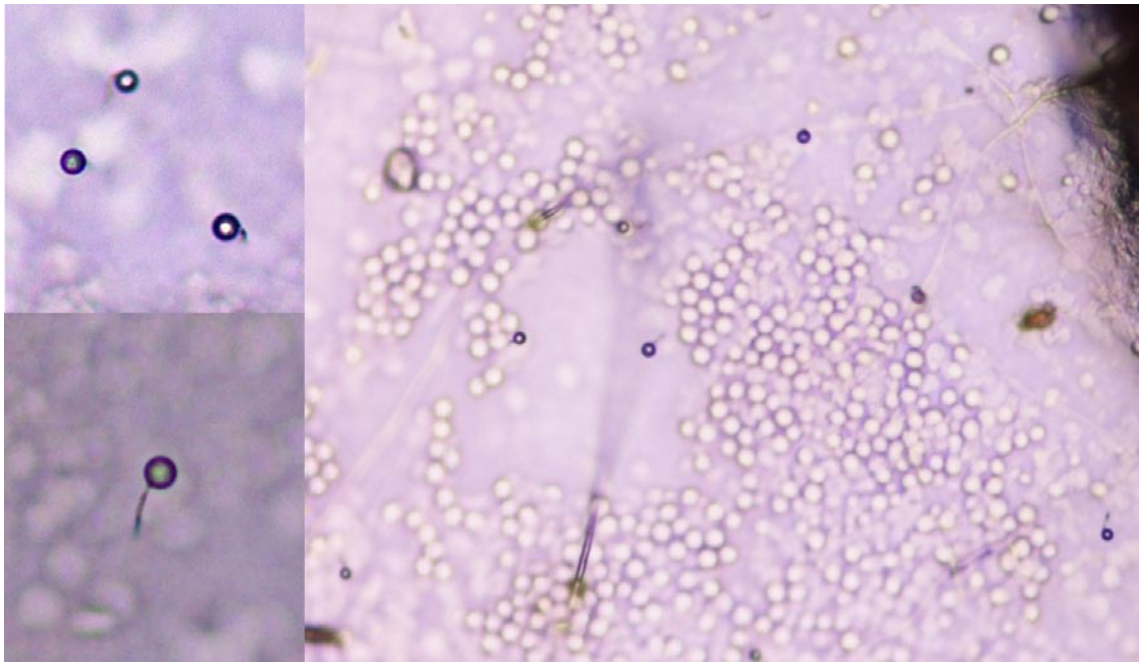
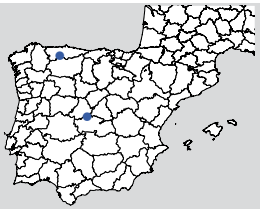


Figure 12 – Fruiting bodies of *Protostelium nocturnum*

***Protostelium okumukumu* Spiegel, Shadwick & Hemmes**



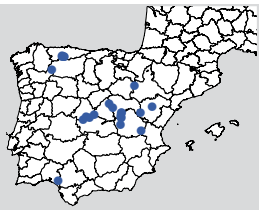
OCCURRENCE: Loc. 11: aerial litter of *Tilia* sp., AS05-104. Loc. 22: aerial litter of *Q. ilex*, AV07-03.

DESCRIPTION: *Sporocarps*- Sporocarps 15-25 µm tall, ballistosporous. Stalk bipartite, with two segments separated by an articulation, apophysis spherical to ovoid present. Spore nearly spherical, (7.2) 9.5-10.5 µm in diam. When intact, the spore and the apophysis flag at the articulation point. The spore is actively shot with the disappearance of the apophysis and only the rigid basal portion of the stalk remains, resembling a "beard stubble". Prespore cells elliptical (Spiegel et al, 2006).

Trophic stages- It can be cultivated on wMY agar at 20-24º C with the yeast *Cryptococcus laurentii* or *Rhodotorula mucilaginosa*. The spores liberate the uninucleate, nonflagellated amoebae typical of the genus *Protostelium*, sensu Spiegel et al (1994). The amoebae contain light orange lipid droplets and may reversibly encyst producing walled, spherical cysts (Discover life).

COMMENTS: It is a rare and recently described species, and it was found only two times during our study.

***Protostelium pyriforme* L. S. Olive & Stoian.**



OCCURRENCE: **Loc. 3:** aerial litter of *Quercus ilex*, AS05-37. **Loc. 5:** aerial litter of *Corylus avellana*, AS05-62; ground litter of *Corylus avellana*, AS05-63. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96. **Loc. 14:** aerial litter of *R. sphaerocarpa,* M06-43. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07. **Loc. 17:** aerial litter of *R. officinalis,* GU06-13; ground litter of *Q. coccifera,* GU06-16. **Loc. 18:** aerial litter of thistle, CU06-03. **Loc. 19:** aerial litter of Gramineae, CU06-05; aerial litter of *Thymus* sp., CU06-07. **Loc. 21:** aerial litter of *Lavandula* sp., M07-17. **Loc. 22:** aerial litter of *Q. ilex*, AV07-03. **Loc. 24:** aerial litter of *Q. pyrenaica*, AV07-13. **Loc. 25:** aerial litter of thistle, TO07-13. **Loc. 29:** aerial litter of Lamiaceae, TE07-30. **Loc. 46:** ground litter of *J. communis*, SO07-04. **Loc. 49:** aerial litter of *Cistus albifolius*, CU07-05. **Loc. 51:** aerial litter of *R. officinalis*, CU07-25. **Loc. 55:** aerial litter of *Thymus* sp., TE07-15. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01. **Loc. 89:** ground litter, H09-35.

DESCRIPTION: *Sporocarps*- Stalk 50-100 µm long, relatively long, narrow, gently tapered, straight to gently curved, with a knob-like apophysis. Spores typically obpyriform or campanulate, 7.5-11.6 X 8.8-12.4 µm, with a small round hilum at the base, often waving in air currents. Prespore cells round to oval (Olive & Stoianovitch, 1969).

Trophic stages- It is mantained in the laboratory on bacteria such as *Escherichia coli* or *Flavobacterium* sp., but it does not survive on yeasts. Protoplasts hyaline, mostly uninucleate and with one contractile vacuole. The amoebae in water produce filose pseudopodia. Cysts typically have a scalloped margin, 8.8-15.2 X 10-17.5 µm (Olive & Stoianovitch, 1969).

COMMENTS: The sporocarps observed are similar in size to the fruiting bodies of *P. mycophaga*. It is a common species, more abundant in the tropics than in temperate regions (Spiegel et al, 2007), but was not very common in this study. It has been previously reported from Germany (Tesmer et al 2005) and Russia (Kosheleva et al 2009).

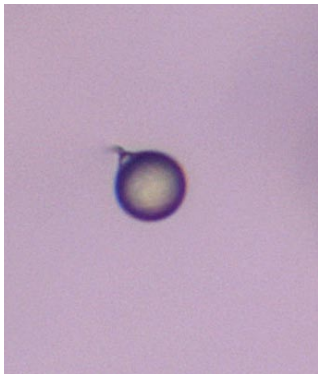
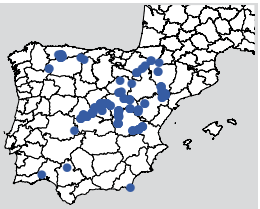


Figure 12 – Fruiting bodies of *Protostelium pyriforme*

***Schizoplasmodiopsis amoeboidea* L. S. Olive & K. D. Whitney**



OCCURRENCE: **Loc. 1:** ground litter of *Pteridium aquilinum*, AS05-6; ground litter of Compositae, AS05-12. **Loc. 2:** ground litter of *Cytisus* sp., AS05-21; ground litter of thistle, AS05-23; bark of *Crataegus monogyna*, AS05-26. **Loc. 4:** ground litter of *Calluna vulgaris*, AS05-42; bark of *Cytisus* sp., AS05-45; aerial litter of *Erica* sp., AS05-48; ground litter of Lamiaceae, AS05-53. **Loc. 5:** aerial litter of *Corylus avellana*, AS05-62. **Loc. 6:** aerial litter of *Fagus sylvatica*, AS05-64; bark of *Fagus sylvatica*, AS05-66. **Loc. 9:** aerial litter of *Cytisus* sp., AS05-83; ground litter of *Cytisus* sp., AS05-84. **Loc. 10:** aerial litter of *Erica arborea*, AS05-90; aerial litter of Poaceae, AS05-91; ground litter of Poaceae, AS05-92; ground litter of *Tilia* sp., AS05-94; bark of *Pinus sylvestris*, AS05-95. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96; aerial litter of *Tilia* sp., AS05-104. **Loc. 12:** ground litter of *Alnus* sp., AS05-114. **Loc. 13:** aerial litter of *Lavandula* sp., M06-31; ground litter of *Lavandula* sp., M06-32; aerial litter of *Thymus* sp., M06-33; ground litter of *Thymus* sp., M06-34; aerial litter of *Q. ilex*, M06-35; ground litter of *G. scorpius*, M06-38. **Loc. 15:** aerial litter of Gramineae, GU06-01; ground litter of Gramineae, GU06-02; aerial litter of Leguminosae, GU06-03; aerial litter of *Lavandula* sp., GU06-05; ground litter of *Lavandula* sp., GU06-06. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07; ground litter of Leguminosae, GU06-10. **Loc. 17:** aerial litter of Gramineae, GU06-11; ground litter of Gramineae, GU06-12; aerial litter of *R. officinalis,* GU06-13; ground litter of *R. officinalis,* GU06-14; aerial litter of *Q. coccifera,* GU06-15; ground litter of *Q. coccifera,*GU06-16. **Loc. 18:** aerial litter of Gramineae, CU06-01; ground litter of Gramineae, CU06-02; ground litter of thistle, CU06-04. **Loc. 19:** aerial litter of Gramineae, CU06-05; ground litter of Gramineae, CU06-06; aerial litter of *Thymus* sp., CU06-07; ground litter of *Thymus* sp., CU06-08. **Loc. 20:** aerial litter of *Lavandula* sp., M07-01; ground litter of *Lavandula* sp., M07-02; aerial litter of *Q. ilex*, M07-05; bark of *Pinus pinea*, M07-10. **Loc. 21:** aerial litter of *Q. ilex*, M07-11; ground litter of *Q. ilex*, M07-12; aerial litter of *C. salvifolius*, M07-13; ground litter of *C. salvifolius*, M07-14; ground litter of *Lavandula* sp., M07-18. **Loc. 22:** aerial litter of Leguminosae, AV07-01; aerial litter of *Q. ilex*, AV07-03; ground litter of *Q. ilex*, AV07-04. **Loc. 23:** aerial litter of *Q. ilex*, TO07-01; aerial litter of *R. sphaerocarpa*, TO07-03; ground litter of *R. sphaerocarpa*, TO07-04; aerial litter of *J. oxycedrus*, TO07-05; ground litter of *J. oxycedrus*, TO07-06; aerial litter of *Lavandula* sp., TO07-07; ground litter of *Lavandula* sp., TO07-08. **Loc. 24:** ground litter of *Rubus* sp., AV07-16; aerial litter of *Q. ilex*, AV07-17; bark of *Q. pyrenaica*, AV07-20. **Loc. 25:** aerial litter of *Q. ilex*, TO07-11; ground litter of thistle, TO07-14; bark of *Q. ilex*, TO07-20. **Loc. 26:** aerial litter of Leguminosae, GU07-05; bark of *J. oxycedrus*, GU07-09; bark of *Q. ilex*, GU07-10. **Loc. 27:** aerial litter of *Juniperus thurifera*, GU07-13; ground litter of *Juniperus thurifera*, GU07-14; aerial litter of *Lavandula* sp., GU07-15; bark of *Juniperus*

sp., GU07-16. **Loc. 28:** aerial litter of Lamiaceae, TE07-19; bark of *Q. faginea*, TE07-27. **Loc. 31:** aerial litter of Cistaceae, TE07-47; ground litter of Cistaceae, TE07-48. **Loc. 32:** aerial litter of *R. officinalis*, Z07-01; ground litter of *R. officinalis*, Z07-02; aerial litter of Compositae, Z07-03; aerial litter of Gramineae, Z07-05; bark of *J. phoenicea*, Z07-09; bark of *R. officinalis*, Z07-10. **Loc. 33:** aerial litter of Gramineae, Z07-13; ground litter of Gramineae, Z07-14; bark of *Pinus halepensis*, Z07-16. **Loc. 34:** ground litter of Gramineae, Z07-26; aerial litter of *P. halepensis*, Z07-27. **Loc. 35:** aerial litter of *Lygeum spartum*, Z07-31; aerial litter of *Arthrocnemum* sp., Z07-33; ground litter of *Arthrocnemum* sp., Z07-34; aerial litter of *Suaeda*, Z07-35; aerial litter of *Suaeda* sp., Z07-36; ground litter of *Salsola* sp., Z07-38. **Loc. 36:** aerial litter of *Lygeum spartum*, HU07-01; ground litter of *Lygeum spartum*, HU07-02; ground litter of Compositae, HU07-06; bark of *R. officinalis*, HU07-09; bark of *J. phoenicea*, HU07-10. **Loc. 37:** ground litter of *Ulex* sp., HU07-14; bark of *Q. faginea*, HU07-19. **Loc. 38:** bark of *Salix* sp., HU07-23. **Loc. 41:** aerial litter of fern, HU07-53. **Loc. 42:** aerial litter of *Rosa* sp., NA07-01. **Loc. 43:** aerial litter of *Q. coccifera*, NA07-16. **Loc. 44:** aerial litter of Gramineae, NA07-21. **Loc. 45:** aerial litter of Leguminosae, NA07-23; ground litter of Leguminosae, NA07-24; aerial litter of *Atriplex halimus*, NA07-33. **Loc. 46:** ground litter of *J. communis*, SO07-04. **Loc. 47:** ground litter of Lamiaceae, SO07-10. **Loc. 48:** ground litter of Lamiaceae, SO07-18; ground litter of *Santolina* sp., SO07-20. **Loc. 49:** aerial litter of *Q. ilex*, CU07-03; ground litter of *Q. ilex*, CU07-04; aerial litter of *Cistus albifolius*, CU07-05; ground litter of *Cistus albifolius*, CU07-06; aerial litter of Gramineae, CU07-07; bark of *Q. ilex*, CU07-09; bark of *J. oxycedrus*, CU07-10. **Loc. 50:** ground litter of Compositae, CU07-14; aerial litter of *Q. ilex*, CU07-15; ground litter of *Q. ilex*, CU07-16; bark of *Pinus* sp., CU07-20. **Loc. 51:** ground litter of Leguminosae, CU07-22; aerial litter of *Thymus* sp., CU07-23; aerial litter of *R. officinalis*, CU07-25; ground litter of *R. officinalis*, CU07-26; aerial litter of Gramineae, CU07-27; ground litter of Gramineae, CU07-28; bark of *Pinus nigra*, CU07-29. **Loc. 52:** aerial litter of *R. officinalis*, CU07-33; ground litter of *R. officinalis*, CU07-34; ground litter of *Q. ilex*, CU07-36; aerial litter of *Q. ilex*, CU07-37; ground litter of Leguminosae, CU07-38. **Loc. 53:** ground litter of *Thymus* sp., CU07-42; ground litter of *Lavandula* sp., CU07-44; aerial litter of Leguminosae, CU07-45; bark of *Crataegus monogyna*, CU07-50. **Loc. 54:** aerial litter of *Cistus* sp., TE07-03; aerial litter of Rosaceae, TE07-06. **Loc. 55:** aerial litter of *Q. ilex*, TE07-09; bark of *Q. ilex*, TE07-17. **Loc. 56:** aerial litter of *Retama sphaerocarpa*, M06-01; ground litter of *Retama sphaerocarpa*, M06-02. **Loc. 57:** aerial litter of Leguminosae, M06-05; ground litter of Leguminosae, M06-06; aerial litter of Gramineae, M06-07; ground litter of Gramineae, M06-08; aerial litter of Leguminosae, M06-09; ground litter of Leguminosae, M06-10; aerial litter of Leguminosae, M06-11; ground litter of Leguminosae, M06-12. **Loc. 58:** aerial litter of Leguminosae, M06-13; ground litter of Leguminosae, M06-14; aerial litter of Leguminosae, M06-15; ground litter of Leguminosae, M06-16. **Loc. 59:** aerial litter of Leguminosae, M06-17; ground litter of Leguminosae, M06-18. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 61:** aerial litter of Leguminosae, M06-25; ground litter of Leguminosae, M06-26. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01; ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 66:** aerial litter of Leguminosae, LE06-03. **Loc. 67:** ground litter of Leguminosae, PA06-02. **Loc. 68:** aerial litter, AL07-01. **Loc. 71:** ground litter, SO06-03. **Loc. 77:** aerial litter of Lavandula sp., CA09-27. **Loc. 87:** bark of *Q. ilex*, H09-29. **Loc. 90:** aerial litter of *Quercus ilex*, SE09-01.

DESCRIPTION: *Sporocarps*- Sporocarps 18-30 µm tall. Stalks 6-14.4 µm, straight, suddenly thinner towards their apex forming a sharp point. Spores nearly spherical, 12-22 µm in diam., single, proportionally big, globose, uninucleate, and non-deciduous, with a minutely punctate surface (Olive & Whitney, 1982). Prespore cells oval to

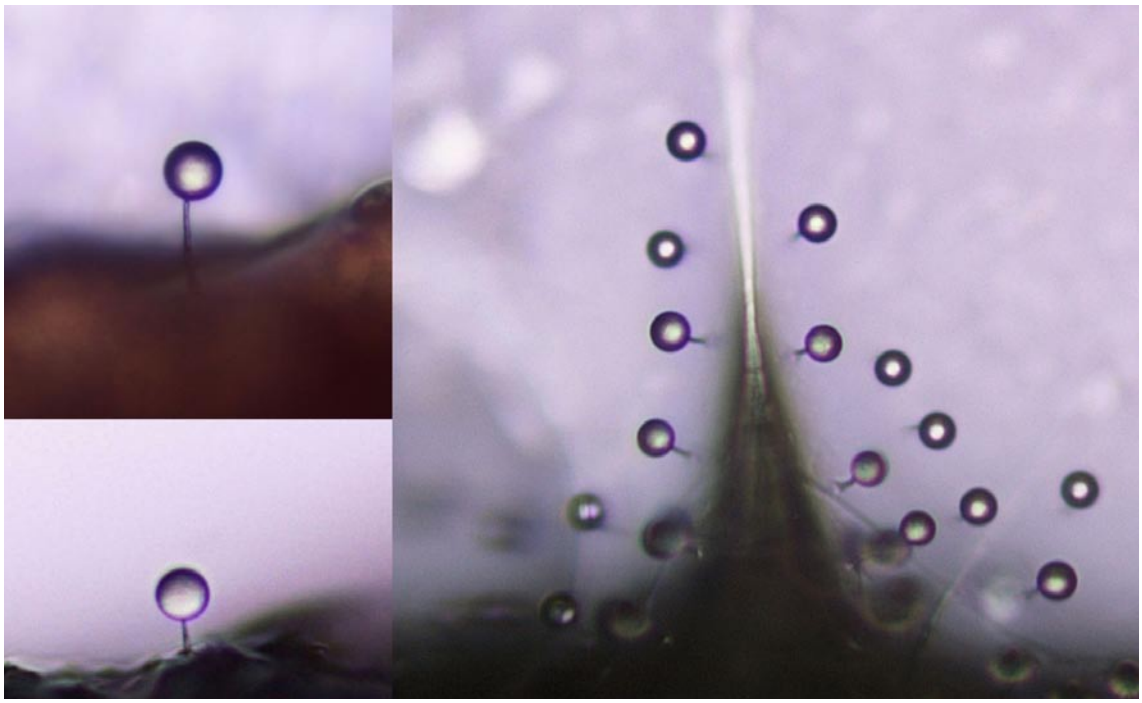


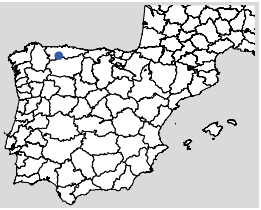
Figure 13 – Fruiting bodies of *Schizoplasmodiopsis amoeboidea*

round in outline.

Trophic stages- It can be cultivated on hay infusion agar, oak bark agar, lactose-yeast extract agar, or wMY agar in association with *Xanthamonas* sp., Malaya bacterium or *Flavobacterium* sp. They produce uninucleate (rarely plurinucleate), thin amoeba. The cysts are typically uninucleate and round, 9-37 µm in diam., or irregular, 7-49 x 12-72 µm (Discover life).

Comments: It is a common species that can be found in many different types of substrate (Spiegel et al, 2007), and was very frequent in our cultures. It has been cited previously in Europe for Germany (Tesmer et al, 2005) and Russia (Kosheleva et al, 2009).

***Schizoplasmodiopsis micropunctata* L. S. Olive & Stoian.**



Occurrence: Loc. 12: ground litter of Lamiaceae, AS05-112.

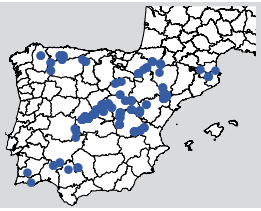
Description: *Sporocarps*- Stalk 9-70 µm, very variable in length, straight, that gets thinner suddenly at the apex forming a hair-like structure at the point of attachment with the spore. The tip of the stalk may be so thin that the spore almost appears as if it is suspended in the air. Spore globose, 11.3-20.6 µm in diam., minutely punctate (Olive & Stoianovitch, 1975). Prespore cells round in outline, formed from single amoebae or segments of the plasmodia.

Trophic stages- It can be cultivated on oak bark agar made with three times the usual amount of oak bark, on supplemented cornmeal agar with half the usual amounts of dextrose and yeast extract, or on wMY agar, grown with bacteria isolated from the original substrate, *Escherichia coli, Flavobacterium* sp., or Florida 20 bacterium as food organisms. The amoebae are thin, uninucleate (sometimes binucleate) and they produce many filose subspeudopodia, They can fuse to produce large, sometimes reticulate plasmodia, but with no nuclear fusion observed. The cysts are round to irregular or reticular, 10-175 x 10-475 µm (Discover life).

Comments: It is very rare but has been encountered worldwide (Spiegel et al, 2007), and it was identified in only one of our cultures. It was also recovered from cultures from Russia (Kosheleva et al 2009).

Figure 14 – Fruiting bodies of *Schizoplasmodiopsis micropunctata*

Schizoplasmodiopsis pseudoendospora L. S. Olive, M. Martin. & Stoian.



OCCURRENCE: **Loc. 1:** aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of Compositae, AS05-11. **Loc. 3:** aerial litter of *Cytisus* sp., AS05-31. **Loc. 4:** ground litter of *Calluna vulgaris*, AS05-42; bark of *Cytisus* sp., AS05-45. **Loc. 6:** ground litter of *Fagus sylvatica*, AS05-65; bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68. **Loc. 10:** ground litter of *Picea abies*, AS05-85; bark of *Picea abies*, AS05-86; ground litter of *Aesculus hippocastanum*, AS05-89; aerial litter of *Erica arborea*, AS05-90; ground litter of *Tilia* sp., AS05-94. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96; ground litter of *Rubus* sp., AS05-97; aerial litter of *Tilia* sp., AS05-104; ground litter of *Tilia* sp., AS05-105. **Loc. 12:** aerial litter of *Rubus* sp., AS05-109; bark of *Alnus* sp., AS05-115; ground litter of Cyperaceae, AS05-117; ground litter of *Rumex* sp., AS05-118. **Loc. 13:** ground litter of Gramineae, M06-30; aerial litter of *Lavandula* sp., M06-31; aerial litter of *Thymus* sp., M06-33; ground litter of *Thymus* sp., M06-34; aerial litter of *Q. ilex*, M06-35; ground litter of *Q. ilex*, M06-36; aerial litter of *G. scorpius*, M06-37; ground litter of *G. scorpius*, M06-38. **Loc. 14:** aerial litter of *C. ladanifer*, M06-39; ground litter of *C. ladanifer*, M06-40; aerial litter of Gramineae, M06-41; ground litter of Gramineae, M06-42; ground litter of *R. sphaerocarpa,* M06-44. **Loc. 15:** aerial litter of Gramineae, GU06-01; ground litter of Gramineae, GU06-02; ground litter of Leguminosae, GU06-04; aerial litter of *Lavandula* sp., GU06-05; ground litter of *Lavandula* sp., GU06-06. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07; ground litter of *Q. coccifera,* GU06-08. **Loc. 17:** aerial litter of Gramineae, GU06-11; aerial litter of *R. officinalis,* GU06-13; ground litter of *R. officinalis,* GU06-14; aerial litter of *Q. coccifera,* GU06-15; ground litter of *Q. coccifera,*GU06-16. **Loc. 18:** ground litter of Gramineae, CU06-02; ground litter of thistle, CU06-04. **Loc. 19:** ground litter of Gramineae, CU06-06; aerial aerial litter of *Thymus* sp., CU06-07. **Loc. 20:** aerial litter of *Lavandula* sp., M07-01; aerial litter of *Q. ilex*, M07-05; ground litter of *Q. ilex*, M07-06. **Loc. 21:** ground litter of *Q. ilex*, M07-12; aerial litter of *C. salvifolius*, M07-13; ground litter of *C. salvifolius*, M07-14; aerial litter of Gramineae, M07-15; aerial litter of *Lavandula* sp., M07-17; ground litter of *Lavandula* sp., M07-18. **Loc. 22:** ground litter of Leguminosae, AV07-02; aerial litter of *Q. ilex*, AV07-03; ground litter of *Q. ilex*, AV07-04; ground litter of *J. oxycedrus*, AV07-08; bark of *Q. ilex*, AV07-09; bark of *J. oxycedrus*, AV07-10. **Loc. 23:** aerial litter of *Q. ilex*, TO07-01; ground litter of *Q. ilex*, TO07-02; ground litter of *R. sphaerocarpa*, TO07-04; aerial litter of *J. oxycedrus*, TO07-05; ground litter of *J. oxycedrus*, TO07-06; aerial litter of *Lavandula* sp., TO07-07; ground litter of *Lavandula* sp., TO07-08. **Loc. 24:** ground litter of *C. ladanifer*, AV07-12; aerial litter of *Q. pyrenaica*, AV07-13; ground litter of *Q. pyrenaica*, AV07-14; ground litter of *Rubus* sp., AV07-16; aerial litter of *Q. ilex*, AV07-17; ground litter of *Q. ilex*, AV07-18; bark of *Q. pyrenaica*, AV07-20. **Loc. 25:** ground litter of *Q. ilex*, TO07-12; ground litter of thistle, TO07-14; aerial litter of *Lavandula* sp., TO07-17; ground litter of *Lavandula* sp., TO07-18; bark of *Q. ilex*, TO07-20. **Loc. 26:** bark of *J. oxycedrus*, GU07-09; bark of *Q. ilex*, GU07-10. **Loc. 27:** aerial litter of *Juniperus thurifera*, GU07-13; aerial litter of *Lavandula* sp., GU07-15; bark of *Juniperus* sp., GU07-16; aerial litter of Leguminosae, GU07-17; bark of *Ulmus* sp., GU07-20. **Loc. 28:** ground litter of Lamiaceae, TE07-20; bark of *Q. faginea*, TE07-27. **Loc. 31:** aerial litter of *Q. coccifera*, TE07-43; ground litter of *Q. coccifera*, TE07-44; ground litter of Lamiaceae, TE07-46; aerial litter of Cistaceae, TE07-47; ground litter of Cistaceae, TE07-48; aerial litter of Gramineae, TE07-49; bark of *Olea europaea*, TE07-52. **Loc. 32:** aerial litter of *R. officinalis*, Z07-01; ground litter of *R. officinalis*, Z07-02; aerial litter of Compositae, Z07-03; ground litter of *Artemisia* sp., Z07-04; ground litter of Gramineae, Z07-06; ground litter of Leguminosae, Z07-08. **Loc. 33:** ground litter of *R. officinalis*, Z07-12; ground litter of Gramineae, Z07-14; aerial litter of *Ephedra* sp., Z07-17; ground litter of *Ephedra* sp., Z07-18. **Loc. 34:** aerial litter of *R. officinalis*, Z07-21; ground litter of *R. officinalis*, Z07-22; bark of *Juniperus* sp., Z07-23; aerial litter of *P. halepensis*, Z07-27. **Loc. 35:** ground litter of *Arthrocnemum* sp., Z07-34; aerial litter of *Suaeda* sp., Z07-36. **Loc. 36:** ground litter of Compositae, HU07-06; bark of *R. officinalis*, HU07-09; bark of *J. phoenicea*, HU07-10. **Loc. 37:** bark of *Q. faginea*, HU07-19. **Loc. 38:** bark of *Salix* sp., HU07-23; aerial litter of *Geum* sp., HU07-27. **Loc. 39:** ground litter of *F. sylvatica*, HU07-32; ground litter of *Quercus* sp., HU07-33. **Loc. 41:** ground litter of fern, HU07-54. **Loc. 42:** ground litter of *Rosa* sp., NA07-02; aerial litter of Gramineae, NA07-07; ground litter of Gramineae, NA07-08. **Loc. 43:** ground litter of *Q. humilis*, NA07-10; ground litter of *Q. coccifera*, NA07-14; ground litter of Gramineae, NA07-15. **Loc. 44:** ground litter of *Q. coccifera*, NA07-20. **Loc. 45:** ground litter of Leguminosae, NA07-24; ground litter of Gramineae, NA07-26; ground litter of Cistaceae, NA07-28; ground litter of *R. officinalis*, NA07-30; aerial litter of *Atriplex halimus*, NA07-33. **Loc. 48:** ground litter of Lamiaceae, SO07-18; ground litter of *Santolina* sp., SO07-20. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; ground litter of *Q. ilex*, CU07-04; aerial litter of *Cistus albifolius*, CU07-05; bark of *Q. ilex*, CU07-09; bark of *J. oxycedrus*, CU07-10. **Loc. 50:** aerial litter of *Thymus* sp., CU07-11; ground litter of Compositae, CU07-14; aerial litter of *Q. ilex*, CU07-15; ground litter of *Q. ilex*, CU07-16; bark of *Q. ilex*, CU07-19; bark of *Pinus* sp., CU07-20. **Loc. 51:** aerial litter of *Thymus* sp., CU07-23; ground litter of *Thymus* sp., CU07-24; ground litter of *R. officinalis*, CU07-26; bark of *Pinus nigra*, CU07-29; bark of *Juniperus* sp., CU07-30. **Loc. 52:** ground litter of *Q. coccifera*, CU07-32; aerial litter of *R. officinalis*, CU07-33; ground litter of *Q. ilex*, CU07-36; ground litter of Leguminosae, CU07-38. **Loc. 53:** aerial litter of *Thymus* sp., CU07-41; ground litter of *Thymus* sp., CU07-42; aerial litter of Leguminosae, CU07-45; bark of *Crataegus monogyna*, CU07-50. **Loc. 54:** aerial litter of *Cistus* sp., TE07-03; aerial litter of *Cistus* sp., TE07-04. **Loc. 55:** aerial litter of *Q. ilex*, TE07-09; ground litter of *Q. ilex*, TE07-10; ground litter of Leguminosae, TE07-14; bark of *Q. ilex*, TE07-17. **Loc. 56:** aerial litter of *Retama sphaerocarpa*, M06-01; ground litter of *Retama sphaerocarpa*, M06-02. **Loc. 57:** aerial litter of Leguminosae, M06-05; aerial litter of Leguminosae, M06-09; ground litter of Leguminosae, M06-10; ground litter of Leguminosae, M06-12. **Loc. 58:** aerial litter of Leguminosae, M06-13; ground litter of Gramineae, M06-16. **Loc. 59:** aerial litter of Leguminosae, M06-17; ground litter of Leguminosae, M06-18. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 61:** ground litter of Leguminosae, M06-26. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01; ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 64:** aerial litter of Leguminosae, LU06-01; ground litter of Leguminosae, LU06-02. **Loc. 65:** aerial litter of *Chamaespartium tridentatum*, LU06-03; ground litter of *Chamaespartium tridentatum*, LU06-04. **Loc. 66:** aerial litter of Leguminosae, LE06-03. **Loc. 67:** aerial litter of Leguminosae, PA06-01; ground litter of Leguminosae, PA06-02. **Loc. 69:** ground litter, SO06-01. **Loc. 71:** ground litter, SO06-03. **Loc. 72:** ground litter, SO06-04. **Loc. 73:** aerial litter of *Acer monspessulanum*, GE08-07. **Loc. 74:** aerial litter of fern, GE08-15; aerial litter of Rosaceae, GE08-17; ground litter of Rosaceae, GE08-18; bark of *Fagus sylvatica*, GE08-20. **Loc. 76:** ground litter of *Q. ilex*, CA09-14. **Loc. 77:** ground litter of *Q. faginea*, CA09-22. **Loc. 78:** ground litter of *Q. ilex*, CA09-38. **Loc. 81:** ground litter of *Cistus* sp., BA09-22; ground litter of Lamiaceae, BA09-28. **Loc. 83:** ground litter of *Cistus* sp., H09-12; ground litter of Gramineae, H09-16. **Loc. 85:** aerial litter of *Lavandula* sp., PO09-17. **Loc. 90:** ground litter of *Q. ilex*, SE09-02. **Loc. 92:** ground litter of *Q. ilex*, CO09-04; aerial litter of *Cistus* sp., CO09-07. **Loc. 93:** ground litter, CO09-12. **Loc. 95:** ground litter, PO09-41. **Loc. 96:** ground litter of Rosaceae, FR08-08.

DESCRIPTION: *Sporocarps*- Stalks proportionally very short, taper evenly to their tip. Spores nearly spherical, 6.2 - 11.5(-13) µm in diam. (Olive, 1967). Prespore

cells are round to irregular in outline.

Trophic stages- They can be cultivated on hay infusion agar or wMY agar with a mixture of Flavobacterium sp. and Escherichia coli, or on an unidentified rod shaped bacterium with which it was isolated (Florida-20). Spore germination liberates a single, uninucleate, elongate amoeba, with long and filose pseudopodia. The amoebae can form plasmodia by nuclear division with no plasmotomy or by fusion of small amoebae. Plasmodia are often branched and anastomosing and may be several millimeters wide. They fragment into uninucleate prespore cells or into cysts (Discover life).

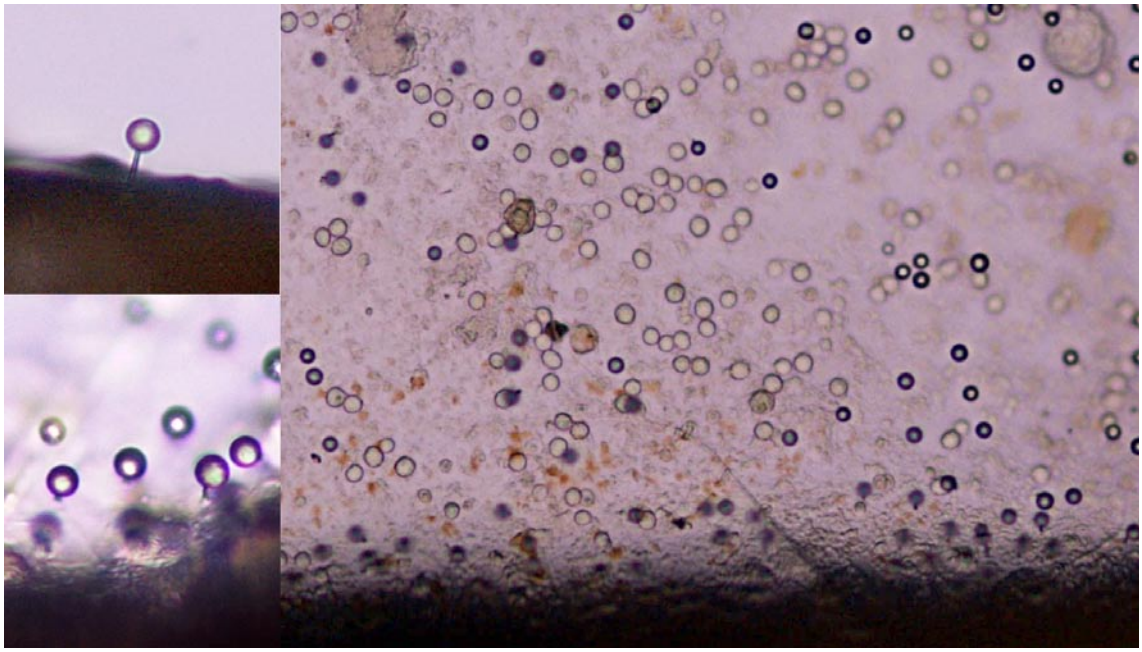


Figure 15 – Fruiting bodies of *Schizoplasmodiopsis pseudoendospora*

Comments: It tends to fruit in big dense patches, and is usually smaller than *S. amoeboidea*. It is one of the smallest but most frequent species, and it is very frequently found in temperate and tropical regions (Spiegel et al, 2007). It has been cited previously in Europe from Germany (Tesmer et al, 2005), Ukraine (Glustchenko et al, 2002) and Russia (Kosheleva et al, 2009). It is one of the most common protostelids worldwide (Spiegel et al, 2007) and it was also very abundant in our samples.

***Schizoplasmodiopsis reticulata* L.S. Olive & Stoian.**

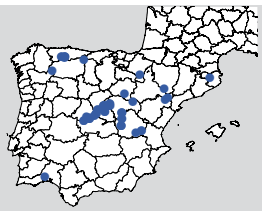
OCCURRENCE: Loc. 31: bark of *Olea europaea*, TE07-52.

DESCRIPTION: *Sporocarps*- Sporocarps very variable in height, 45-110 µm tall. Stalks 38-90 µm long, gracile. Spores spherical, 7-20 µm in diam., with a reticulum of ridges (Olive & Stoianovitch, 1975). Prespore cells round, formed from single amoebae or segments of plasmodia.

Trophic stages- It can be cultivated on oak-bark agar (Olive, 1975a) or wMY agar in association with bacteria isolated from its original substrate. The spores produce a single uninucleate amoeba, which is branched with numerous, often branched filose subpseudopodia. They can fuse to produce large plasmodia but no nuclear fusion is observed. The cysts are globose to oblong or irregular, 7-32 x 7-58 µm (Discover life).

COMMENTS: This is a relatively rare species, but it is widespread. It occurs in situations wherever *S. vulgare* is likely to be found (Spiegel et al, 2007). It was found only once during our study.

***Schizoplasmodiopsis vulgaris* L. S. Olive & Stoian.**



OCCURRENCE: **Loc. 1:** aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; ground litter of Compositae, AS05-12. **Loc. 6:** ground litter of *Fagus sylvatica*, AS05-65. **Loc. 12:** ground litter of *Rubus* sp., AS05-110; ground litter of *Equisetum* sp., AS-121. **Loc. 13:** aerial litter of *Lavandula* sp., M06-31; ground litter of *Lavandula* sp., M06-32; aerial litter of *Q. ilex*, M06-35; ground litter of *G. scorpius*, M06-38. **Loc. 14:** aerial litter of Gramineae, M06-41; aerial litter of *R. sphaerocarpa,* M06-43. **Loc. 15:** aerial litter of Leguminosae, GU06-03. **Loc. 17:** ground litter of Gramineae, GU06-12; aerial litter of *R. officinalis,* GU06-13; aerial litter of *Q. coccifera,* GU06-15. **Loc. 19:** aerial litter of Gramineae, CU06-05; ground litter of *Thymus* sp., CU06-08. **Loc. 20:** aerial litter of *Lavandula* sp., M07-01; aerial litter of *R. sphaerocarpa*, M07-03; ground litter of *R. sphaerocarpa*, M07-04. **Loc. 21:** aerial litter of *Q. ilex*, M07-11. **Loc. 22:** ground litter of *Q. ilex*, AV07-04. **Loc. 23:** ground litter of *R. sphaerocarpa*, TO07-04; aerial litter of *Lavandula* sp., TO07-07. **Loc. 24:** aerial litter of *Q. pyrenaica*, AV07-13. **Loc. 25:** aerial litter of *Q. ilex*, TO07-11; bark of *Q. ilex*, TO07-20. **Loc. 27:** aerial litter of *Juniperus thurifera*, GU07-13. **Loc. 31:** bark of *Olea europaea*, TE07-52. **Loc. 32:** aerial litter of *R. officinalis*, Z07-01. **Loc. 36:** ground litter of Compositae, HU07-06. **Loc. 45:** aerial litter of *Atriplex halimus*, NA07-33. **Loc. 47:** ground litter of Lamiaceae, SO07-10. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; aerial litter of *Cistus albifolius*, CU07-05; ground litter of Gramineae, CU07-08. **Loc. 50:** ground litter of *Q. ilex*, CU07-16. **Loc. 51:** ground litter of *R. officinalis*, CU07-26; bark of *Juniperus* sp., CU07-30. **Loc. 56:** aerial litter of *Retama sphaerocarpa*, M06-01; ground litter of *Retama sphaerocarpa*, M06-02. **Loc. 57:** ground litter of Leguminosae, M06-06; aerial litter of Leguminosae, M06-11. **Loc. 58:** ground litter of Leguminosae, M06-14; ground litter of Gramineae, M06-16. **Loc. 59:** ground litter of Leguminosae, M06-18. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 61:** ground litter of Leguminosae, M06-26. **Loc. 62:** ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 66:** aerial litter of Leguminosae, LE06-03. **Loc. 74:** bark of *Quercus* sp., GE08-19. **Loc. 87:** aerial litter of compositae, H09-27.

DESCRIPTION: *Sporocarps*- Stalk relatively thick and very variable in length, 9-70 µm long, not tappering to one poing. Spores, 8-16 (-37) µm in diam., nearly spherical and coarse, with low ridges formed by a reticulum of spore wall thickenings that appear as slight bumps (Olive & Stoianovitch, 1975). Prespore cells circular in outline, and usually many of them are formed simultaneously due to fragmentation of the plasmodium.

Trophic stages- It can be grown on hay infusion agar, oak bark agar, lactose-yeast agar or wMY agar on association with pre-grown Florida. Each spore gives rise to a single, thin, branched amoeba with filose subpseudopodia and several contractilve vacuoles. They can fuse to produce large plasmodia with no nuclear fusion observed. The cysts are round to irregular, uninucleate to plurinucleate, 5-66 x 7-300 µm (Discover life).

COMMENTS: This species has been cited in Europe for England (Olive 1975b), Germany (Tesmer et al, 2005), and Russia (Kosheleva et al, 2009). It is a common species worldwide, and in cool, moist habitats, it is often one of the only species encountered (Spiegel et al, 2007). It was quite common in our cultures from the Iberian peninsula.

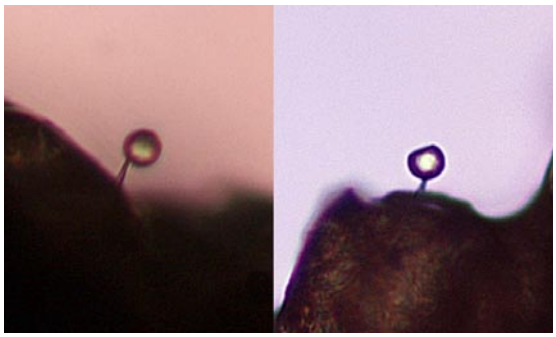
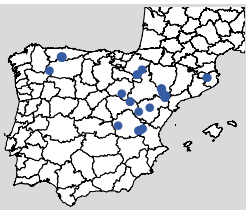


Figure 15 – Fruiting bodies of *Schizoplasmodiopsis vulgaris*

***Schizoplasmodium cavostelioides* L. S. Olive & Stoian.**



OCCURRENCE: **Loc. 1:** ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of Compositae, AS05-11. **Loc. 6:** bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68. **Loc. 12:** aerial litter of *Rubus* sp., AS05-109. **Loc. 20:** ground litter of *R. sphaerocarpa*, M07-04. **Loc. 21:** aerial litter of Gramineae, M07-15. **Loc. 27:** bark of *Juniperus* sp., GU07-16; ground litter of Leguminosae, GU07-18. **Loc. 29:** ground litter of Gramineae, TE07-33. **Loc. 32:** ground litter of *R. officinalis*, Z07-02; bark of *J. phoenicea*, Z07-09. **Loc. 33:** bark of *R. officinalis*, Z07-15; bark of *Pinus halepensis*, Z07-16. **Loc. 35:** aerial litter of *Arthrocnemum* sp., Z07-33. **Loc. 36:** aerial litter of *Lygeum spartum*, HU07-01; bark of *R. officinalis*, HU07-09; bark of *J. phoenicea*, HU07-10. **Loc. 44:** ground litter of *Q. coccifera*, NA07-20. **Loc. 45:** ground litter of Leguminosae, NA07-24; aerial litter of Cistaceae, NA07-27. **Loc. 47:** ground litter of Leguminosae, SO07-16. **Loc. 49:** bark of *J. oxycedrus*, CU07-10. **Loc. 51:** ground litter of Leguminosae, CU07-22; ground litter of Gramineae, CU07-28; bark of *Pinus nigra*, CU07-29; bark of *Juniperus* sp., CU07-30. **Loc. 52:** aerial litter of *Q. ilex*, CU07-37. **Loc. 54:** aerial litter of *Cistus* sp., TE07-03. **Loc. 62:** ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 74:** aerial litter of *Fagus sylvatica*, GE08-11; aerial litter of *Castanea sativa*, GE08-13.

DESCRIPTION: *Sporocarps*- Sporocarps ballistosporous. Stalk 4.3-8 µm long, relatively short, thick, with a a distinct cup-shaped apophysis. Spore almost spherical, 11-20.5 µm in diam., relatively big, smooth, typically multinucleated, attached to the

stalk by a ring-shaped hilum that fits the apophysis (Olive & Stoianovitch, 1966b).

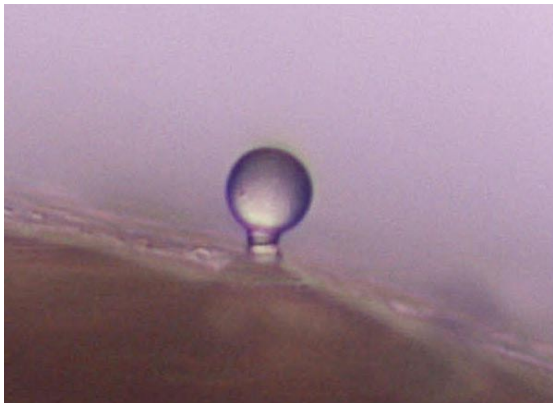


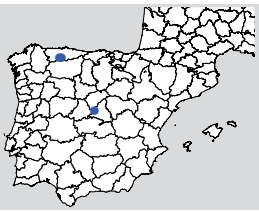
Figure 16 – Fruiting bodies of *Schizoplasmodium cavostelioides.*

Just before the spore discharge, a swelling of the sheath, interpreted as a gas bubble, appears. Prespore cells are round from above and hat-shaped from the side.

Trophic stages- It grows well on hay infusion agar with its food organism which is a cream-colored yeast contaminant (Kitani). It forms plasmodia that can be reticulate, eventually fragmenting into few to many multinucleate prespore cells. The cysts are very variable in size and shape, 12-30 x 13.5-50 µm. (Discover life).

COMMENTS: It is a fairly common species in temperate areas and also common in the tropics (Spiegel et al, 2007), and was not uncommon in present study. This species has been reported previously from Germany (Tesmer et al, 2005).

***Soliformovum expulsum* (L. S. Olive & Stoian.) Spiegel**



OCCURRENCE: Loc. 6: bark of *Fagus sylvatica*, AS05-66. Loc. 11: aerial litter of *Rubus* sp., AS05-96. Loc. 61: ground litter of *Leguminosae*, M06-26.

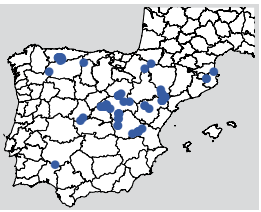
DESCRIPTION: *Sporocarps*- Sporocarps 32.5-45 µm tall, ballistosporous. Stalk bipartite with a broadly tapered basal section and a uniformly thin apical section, usually sharply reflexed at the junction of the two sections. Spore spherical, 11.3-17.4 µm diam., usually two, proportionally big, forcibly discharged with the disappearance of the stalk (Olive & Stoianovitch, 1981). Prespore cells "fried egg" shaped.

Trophic stages- It can be cultivated on oak bark agar (at pH 7) with *Xanthomonas* sp. as food organism. It produces amoeboid and non-flagellate cells, usually flabellate during migration. They are typically uninucleate, and occasionally binucleate. Their nucleolus is irregular and often multi-lobed. The cysts are round, 9-33.6 µm diam., oval, or irregular in shape, 7.2-22.8 X 9.6-26.4 µm, uninucleate. The nucleolus

of the cyst nucleus generally has a more regular shape than that of amoeboid cells (Discover life).

COMMENTS: It was originally described as *Protostelium expulsum* L. S. Olive & Stoian.. It has been found only once during our study. It is not uncommon and somewhat more abundant in the tropics than in temperate habitats (Spiegel et al, 2007).

***Soliformovum irregulare* (L. S. Olive & Stoian.) Spiegel**



OCCURRENCE: **Loc. 1:** aerial litter of Compositae, AS05-11. **Loc. 2:** aerial litter of *Cytisus* sp., AS05-20; bark of *Crataegus monogyna*, AS05-26. **Loc. 3:** aerial litter of *Cytisus* sp., AS05-31; aerial litter of Lamiaceae, AS05-39. **Loc 4:** aerial litter of *Mentha* sp., AS05-52. **Loc. 5:** ground litter of *Corylus avellana*, AS05-63. **Loc. 6:** aerial litter of *Erica* sp., AS05-68. **Loc. 8:** aerial litter of Poaceae, AS05-77. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96; aerial litter of Compositae, AS05-102; aerial litter of *Tilia* sp., AS05-104. **Loc. 12:** ground litter of Lamiaceae, AS05-112; ground litter of *Alnus* sp., AS05-114; ground litter of Cyperaceae, AS05-117; ground litter of *Rumex* sp., AS05-118; ground litter of *Equisetum* sp., AS-121. **Loc. 13:** aerial litter of *Thymus* sp., M06-33; aerial litter of *Q. ilex*, M06-35. **Loc. 14:** aerial litter of *C. ladanifer*, M06-39; ground litter of Gramineae, M06-42. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07. **Loc. 17:** aerial litter of Gramineae, GU06-11; ground litter of Gramineae, GU06-12; aerial litter of *R. officinalis,* GU06-13; aerial litter of *Q. coccifera,* GU06-15. **Loc. 19:** aerial aerial litter of *Thymus* sp., CU06-07; ground litter of *Thymus* sp., CU06-08. **Loc. 24:** aerial litter of *Q. pyrenaica*, AV07-13. **Loc. 25:** aerial litter of *Lavandula* sp., TO07-17. **Loc. 26:** ground litter of *Q. coccifera*, GU07-08. **Loc. 27:** bark of *Ulmus* sp., GU07-20. **Loc. 28:** aerial litter of Lamiaceae, TE07-19; ground litter of Lamiaceae, TE07-20. **Loc. 29:** ground litter of Lamiaceae, TE07-31. **Loc. 31:** ground litter of Cistaceae, TE07-48; aerial litter of Gramineae, TE07-49; bark of *Olea europaea*, TE07-52. **Loc. 32:** aerial litter of *R. officinalis*, Z07-01; ground litter of *R. officinalis*, Z07-02; aerial litter of Compositae, Z07-03. **Loc. 33:** ground litter of Gramineae, Z07-14. **Loc. 35:** ground litter of *Lygeum spartum*, Z07-32; ground litter of *Arthrocnemum* sp., Z07-34; aerial litter of *Suaeda* sp., Z07-36. **Loc. 36:** ground litter of Compositae, HU07-06. **Loc. 41:** aerial litter of fern, HU07-53. **Loc. 43:** aerial litter of *Q. coccifera*, NA07-16. **Loc. 47:** ground litter of Lamiaceae, SO07-10. **Loc. 48:** ground litter of Lamiaceae, SO07-18; aerial litter of *Santolina* sp., SO07-19; ground litter of *Santolina* sp., SO07-20; aerial litter of Gramineae, SO07-22. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; ground litter of *Cistus albifolius*, CU07-06. **Loc. 50:** aerial litter of Compositae, CU07-13. **Loc. 51:** ground litter of *Thymus* sp., CU07-24; aerial litter of *R. officinalis*, CU07-25; ground litter of *R. officinalis*, CU07-26; bark of *Pinus nigra*, CU07-29. **Loc. 52:** aerial litter of *R. officinalis*, CU07-33. **Loc. 57:** aerial litter of Leguminosae, M06-05; aerial litter of Gramineae, M06-07. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01. **Loc. 67:** ground litter of Leguminosae, PA06-02. **Loc. 73:** aerial litter of *Acer monspessulanum*, GE08-07. **Loc. 74:** ground litter of *Castanea sativa*, GE08-14; ground litter of fern, GE08-16. **Loc. 82:** aerial litter of Lamiaceae, H09-07.

DESCRIPTION: *Sporocarps*- Spores nearly spherical, 13.9-22.5 µm diam. Stalks 30-127 µm long, proportionally long, straight, gently tapered with a hastate apophysis (Olive & Stoianovitch, 1969). Spore deciduous,can adhere to the side of the stalk after falling, becomes "American football"-shaped when dryed. Prespore cells "fried egg" shaped.

Trophic stages- Amoebae typically uninucleate (but also plurinucleate). The nucleolus is divided into many small, phase dense subunits, and the nucleus may be irregular in outline. The amoebae are very thin, almost invisible on the agar surface when viewed with bright field optics, and slightly bigger than those of *S. expulsum*. The amoebae have numerous, small contractile vacuoles and many food vacuoles when they are feeding. They are typically flabellate when migrating, with a broad lamellopodial front. There are

numerous acutely pointed subseudopodia which can become quite elongated under very moist conditions. Motility appears to be solely by pseudopodial crawling and gliding has not been observed (Spiegel et al, 1994).

COMMENTS: It is one of the most common species in temperate areas and worldwide (Spiegel et al, 2007), and it was also quite common in our cultures. In Europe, this species have been cited from Germany (Tesmer et al, 2005) and Russia (Kosheleva et al 2009).

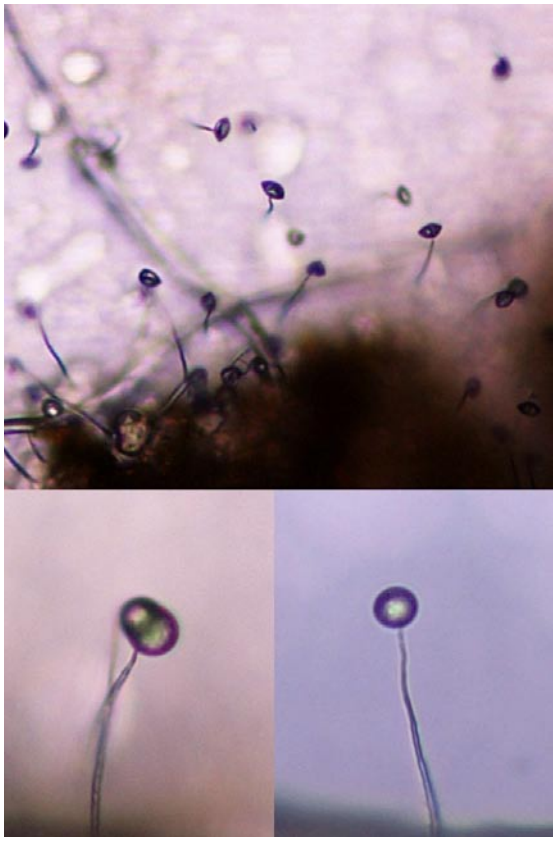
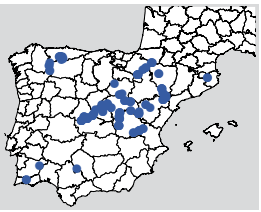


Figure 17 – Fruiting bodies of *Soliformovum irregulare*

Tychosporium acutostipes **Spiegel, D. L. Moore & J. Feldman**



OCCURRENCE: **Loc. 1:** ground litter of *Pteridium aquilinum*, AS05-6. **Loc. 2:** ground litter of *Cytisus* sp., AS05-21. **Loc. 3:** aerial litter of Lamiaceae, AS05-39. **Loc. 4:** ground litter of Lamiaceae, AS05-53. **Loc. 6:** ground litter of fern, AS05-67; aerial litter of *Erica* sp., AS05-68. **Loc. 9:** ground litter of *Gentiana lutea*, AS05-80. **Loc. 10:** ground litter of *Picea abies*, AS05-85. **Loc. 11:** aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; ground litter of Compositae, AS05-103. **Loc. 12:** aerial litter of Lamiaceae, AS05-111; ground litter of Lamiaceae, AS05-112; ground litter of *Rumex* sp., AS05-118. **Loc. 13:** ground litter of Gramineae, M06-30; aerial litter of *Lavandula* sp., M06-31; ground litter of *Lavandula* sp., M06-32; aerial litter of *Thymus* sp., M06-33; ground litter of *Thymus* sp., M06-34; aerial litter of *Q. ilex*, M06-35; ground litter of *G. scorpius*, M06-38. **Loc. 14:** ground litter of Gramineae, M06-42; aerial litter of *R. sphaerocarpa,* M06-43. **Loc. 15:** ground litter of Gramineae, GU06-02; aerial litter of Leguminosae, GU06-03; ground litter of Leguminosae, GU06-04; aerial litter of *Lavandula* sp., GU06-05; ground litter of *Lavandula* sp., GU06-06. **Loc. 16:** aerial litter of *Q. coccifera,* GU06-07; ground litter of *Q. coccifera,* GU06-08. **Loc. 17:** aerial litter of Gramineae, GU06-11; ground litter of Gramineae, GU06-12; aerial litter of *R. officinalis,* GU06-13; ground litter of *R. officinalis,* GU06-14; aerial litter of *Q. coccifera,* GU06-15. **Loc. 18:** ground litter of Gramineae, CU06-02; ground litter of thistle, CU06-04. **Loc. 19:** aerial litter of Gramineae, CU06-05; ground litter of Gramineae, CU06-06; aerial litter of *Thymus* sp., CU06-07; ground litter of *Thymus* sp., CU06-08. **Loc. 20:** aerial litter of *Lavandula* sp., M07-01; ground litter of *Lavandula* sp., M07-02. **Loc. 21:** ground litter of *C. salvifolius*, M07-14; aerial litter of *Lavandula* sp., M07-17; ground litter of *Lavandula* sp., M07-18. **Loc. 22:** aerial litter of Leguminosae, AV07-01; bark of *Q. ilex*, AV07-09. **Loc. 23:** aerial litter of *Q. ilex*, TO07-01; aerial litter of *R. sphaerocarpa*, TO07-03; ground litter of *R. sphaerocarpa*, TO07-04; ground litter of *J. oxycedrus*, TO07-06. **Loc. 24:** aerial litter of *C. ladanifer*, AV07-11; aerial litter of *Q. pyrenaica*, AV07-13; ground litter of *Rubus* sp., AV07-16. **Loc. 25:** aerial litter of thistle, TO07-13; ground litter of thistle, TO07-14; ground litter of *C. ladanifer*, TO07-16; aerial litter of *Lavandula* sp., TO07-17; ground litter of *Lavandula* sp., TO07-18. **Loc. 26:** aerial litter of *Santolina* sp.,

GU07-03; aerial litter of *Thymus* sp., GU07-07; ground litter of *Q. coccifera*, GU07-08. **Loc. 27:** ground litter of Gramineae, GU07-12; ground litter of Leguminosae, GU07-18; bark of *Ulmus* sp., GU07-20. **Loc. 28:** aerial litter of Lamiaceae, TE07-19; ground litter of Lamiaceae, TE07-20; ground litter of *Q. faginea*, TE07-26. **Loc. 29:** ground litter of Lamiaceae, TE07-31. **Loc. 31:** ground litter of *Q. coccifera*, TE07-44; aerial litter of Lamiaceae, TE07-45; aerial litter of Cistaceae, TE07-47; aerial litter of Gramineae, TE07-49. **Loc. 32:** aerial litter of Compositae, Z07-03; ground litter of *Artemisia* sp., Z07-04. **Loc. 33:** ground litter of Gramineae, Z07-14. **Loc. 34:** ground litter of Gramineae, Z07-26. **Loc. 35:** aerial litter of *Lygeum spartum*, Z07-31. **Loc. 36:** aerial litter of *Lygeum spartum*, HU07-01; ground litter of *Lygeum spartum*, HU07-02; aerial litter of Lamiaceae, HU07-03; ground litter of Compositae, HU07-06; bark of *J. phoenicea*, HU07-10. **Loc. 37:** ground litter of *Buxus sempervirens*, HU07-12; bark of *J. communis*, HU07-20. **Loc. 41:** aerial litter of fern, HU07-53; ground litter of fern, HU07-54. **Loc. 42:** aerial litter of *Rosa* sp., NA07-01; aerial litter of Gramineae, NA07-07. **Loc. 43:** ground litter of Leguminosae, NA07-12; aerial litter of Gramineae, NA07-13. **Loc. 44:** ground litter of *Q. coccifera*, NA07-20. **Loc. 45:** aerial litter of Leguminosae, NA07-23; ground litter of Leguminosae, NA07-24; aerial litter of Gramineae, NA07-25; ground litter of Gramineae, NA07-26; ground litter of Cistaceae, NA07-28; ground litter of *R. officinalis*, NA07-30; ground litter of Compositae, NA07-32; aerial litter of *Atriplex halimus*, NA07-33. **Loc. 47:** aerial litter of Lamiaceae, SO07-09. **Loc. 48:** ground litter of Lamiaceae, SO07-18; ground litter of *Santolina* sp., SO07-20. **Loc. 49:** aerial litter of *R. officinalis*, CU07-01; ground litter of *R. officinalis*, CU07-02; ground litter of *Q. ilex*, CU07-04; aerial litter of *Cistus albifolius*, CU07-05; aerial litter of Gramineae, CU07-07; ground litter of Gramineae, CU07-08; bark of *J. oxycedrus*, CU07-10. **Loc. 50:** aerial litter of *Thymus* sp., CU07-11; ground litter of *Thymus* sp., CU07-12. **Loc. 51:** aerial litter of Leguminosae, CU07-21; ground litter of Leguminosae, CU07-22; aerial litter of *Thymus* sp., CU07-23; ground litter of *Thymus* sp., CU07-24; aerial litter of *R. officinalis*, CU07-25. **Loc. 52:** aerial litter of *Q. ilex*, CU07-35; ground litter of *Q. ilex*, CU07-36. **Loc. 53:** ground litter of *Thymus* sp., CU07-42; ground litter of Leguminosae, CU07-46. **Loc. 54:** aerial litter of *Cistus* sp., TE07-03. **Loc. 55:** aerial litter of *Q. ilex*, TE07-09; ground litter of *Q. ilex*, TE07-10; ground litter of Leguminosae, TE07-14; aerial litter of *Thymus* sp., TE07-15; ground litter of *Thymus* sp.,TE07-16. **Loc. 56:** aerial litter of *Retama sphaerocarpa*, M06-01; ground litter of *Retama sphaerocarpa*, M06-02. **Loc. 57:** aerial litter of Leguminosae, M06-09; ground litter of Leguminosae, M06-10; ground litter of Leguminosae, M06-12. **Loc. 58:** ground litter of Leguminosae, M06-14. **Loc. 60:** ground litter of Leguminosae, M06-22. **Loc. 62:** aerial litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-01; ground litter of *Epilobium hirsutum* and *Lithrum salicaria*, O06-02. **Loc. 63:** aerial litter of Gramineae, LE06-01; ground litter of Gramineae, LE06-02. **Loc. 64:** ground litter of Leguminosae, LU06-02. **Loc. 72:** ground litter, SO06-04. **Loc. 74:** aerial litter of fern, GE08-15; ground litter of fern, GE08-16. **Loc. 84:** ground litter of *Q. ilex*, PO09-04. **Loc. 86:** ground litter of *Cistus* sp., PO09-22; ground litter of *Q. suber*, PO09-28. **Loc. 92:** ground litter of *Cistus* sp., CO09-08.

Description: *Sporocarps*- Stalks (6.9-)35-64 µm long, with a somewhat undulate surface, stiff, gradually thinner towards their apex, sharp-poited, undulated. Spores turbinate to nearly spherical, 8.0-12.5 µm in diam, uninucleate, relatively indeciduous (Spiegel et al, 1995), sometimes "American football"-shaped when dryed. In air currents spores can incline to one side, but remaining attached to the stalk. Prespore cells ellipsoidal.

Trophic stages- It grows and fruits

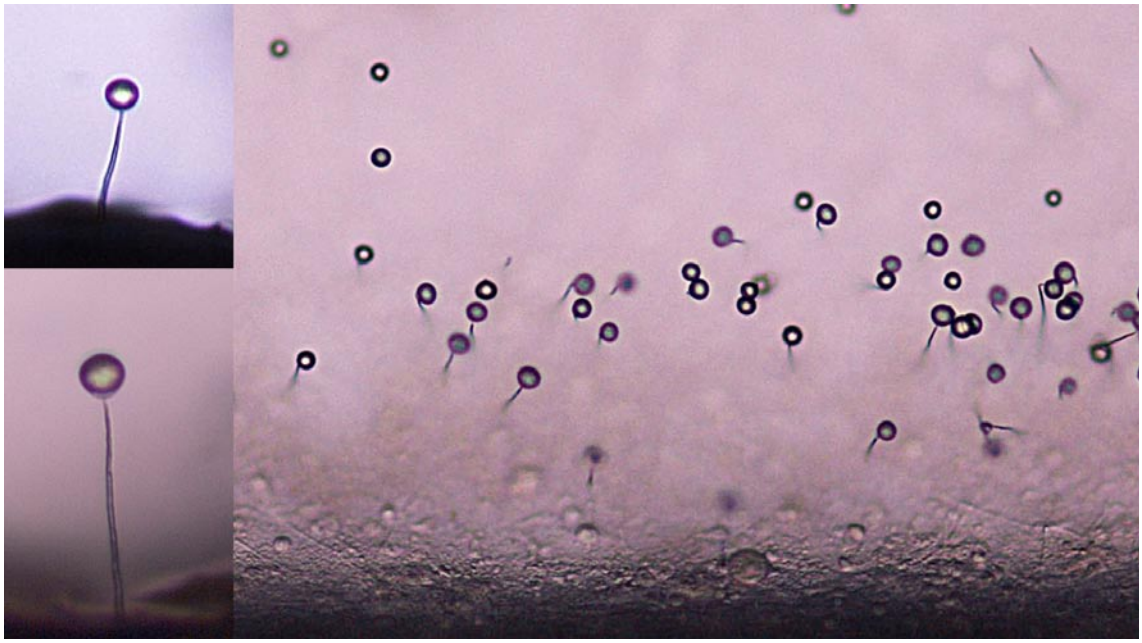


Figure 18 – Fruiting bodies of *Tychosporium acutostipes*

well on wMY agar with either Fla-20 or *Flavobacterium* sp. as its food source. The amoebae are typically uninucleate and unpigmented, though they may become plurinucleate in older cultures (Discover life).

Comments: It is usually a relatively uncommon species, but is found worldwide (Spiegel et al, 2007). It was abundant in our cultures. This species has been cited for Germany (Tesmer et al. 2005) and Russia (Kosheleva et al 2009).

Acknowledgements
We wish to thank Fátima Durán for technician work, and Fred Spiegel, Lora Lindley Shadwick, John Shadwick, Matt Brown and George Ndiritu for all their kind suggestions. This work has been supported by the Research Projects CGL2005-00320/BOS and CGL2008-00720/BOS of the Ministry of Science and Innovation of Spain.

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Capítulo 5: Filogeografía de *Badhamia melanospora*

Tras realizar el estudio de los factores que influyen en la distribución de las amebas protosteloides en la Península Ibérica, quisimos aprovechar el potencial de los mixomicetes para investigar los patrones geográficos a nivel infraespecífico en los eumicetozoos. El uso de caracteres moleculares nos permitió estudiar con mucha más sensibilidad la variabilidad que existe dentro de una misma morfoespecie, y comprobar si existen cepas con una distribución más o menos restringida, o por el contrario "todo está en todas partes". Los mixomicetes poseen una gran ventaja frente a la mayor parte de los protistas incluyendo las amebas protosteloides, y es que las muestras pueden conservarse como material de herbario. Las esporas, debido probablemente a su gran dureza, son capaces de sobrevivir y ser viables incluso pasados muchos años guardadas en el herbario después de su recolección (Smith, 1929). Esto hace que sea mucho más sencillo poder obtener un amplio número de muestras con distinta procedencia geográfica, por lo que los mixomicetes podrían convertirse en un modelo de gran utilidad para realizar estudios filogeográficos y biogeográficos en protistas. El fruto de este estudio se expone en el siguente artículo en preparación:

Aguilar M, Fiore-Donno A-M, Lado C, Cavalier-Smith T. (2011). A geographically structured complex of genetically, morphologically and ecologically diverse cryptic species in Myxomycetes. (in prep.)

Resumen: La dispersión restringida parece haber sido un factor importante para la diferenciación genética en el mixomicete *Badhamia melanospora,* un taxón en el que no todo está en todas partes. Se ha secuenciado aproximadamente un tercio de la subunidad pequeña del ribosoma en 125 ejemplares procedentes de 91 localidades repartidas por todo el área de distribución conocida de la especie. La mayoría de las muestras han sido recolectadas en América del Norte y América del Sur, donde la especie es más común, y a las mismas latitudes a ambos lados del Ecuador. Mediante el uso de inferencia bayesiana y parsimonia estadística se han podido distinguir dos grupos de ribotipos que coinciden con esta separación geográfica. Uno comprende todas las poblaciones de Argentina y Chile (grupo A) y el otro, que constituye un clado divergente, está formado por poblaciones de Norteamérica y la mayoría de las poblaciones de otras partes del mundo (grupo B). Los dos grupos genéticos diferenciados A y B son congruentes con diferencias morfológicas en la ornamentación y tamaño de las esporas y también muestran distintas preferencias ecológicas. Los modelos de nicho ambiental para el ribotipo A predicen presencias en areas restringidas cerca de la costa, y para el grupo B, a pesar de ser geneticamente menos diverso, un nicho más

amplio que alcanza áreas más alejadas de la costa. Se puede concluir que *B. melanospora* constituye un complejo de especies formado por al menos dos criptoespecies. Estos resultados son congruentes con la hipótesis de que algunas morfoespecies de mixomicetes (Myxogastria: Amoebozoa) son complejos de líneas clonales apomícticas geográficamente restringidas. Como la mayoría de las muestras recolectadas en el viejo mundo fueron encontradas sobre plantas suculentas (*Opuntia*, *Agave*) introducidas desde Norteamérica, nuestros resultados también sugieren que *B. melanospora* podría haber sido introducida junto con sus plantas portadoras.

NOTA: El material suplementario correspondiente a este capítulo se encuentra en el Apéndice 2 situado al final de la memoria.

A geographically structured complex of genetically, morphologically and ecologically diverse cryptic species in Myxomycetes

María Aguilar, Anne-Marie Fiore-Donno, Carlos Lado & Tom Cavalier-Smith

Restricted dispersion seems to have been a major factor leading to genetic differentiation in the myxomycete slime mould *Badhamia melanospora*, a taxon in which everything is not everywhere. We sequenced ca. a third of the small-subunit ribosomal gene of 125 specimens from 91 different localities distributed along all known distribution area of the species. Most samples were collected in North and South America, where the species is more common, and at the same opposite latitudes. Using Bayesian inference and statistical parsimony, two groups of ribotypes that match this geographical separation can be distinguished. One comprises all populations from Argentina and Chile (group A), and the other, which constitutes a well defined divergent clade, is formed by populations from North America and most populations from other parts of the world (group B). The two genetically distinct groups A and B are congruent with morphological differences in the ornamentation and size of the spores, and they also have different ecological preferences. The environmental niche model for ribotype group A predicts presence in restricted areas near the coast, and group B, despite being genetically less diverse, has a broader niche that reaches inland areas. It can be concluded that *B. melanospora* constitutes a species complex formed by at least two cryptic species that may have diverged allopatrically. These results are consistent with the hypothesis that some morphospecies of myxomycetes (Myxogastria: Amoebozoa) are complexes of geographically restricted apomictic clonal lines. As most specimens collected in the Old World were found growing on succulent plants (*Opuntia*, *Agave*) introduced from North America, our results also suggest that they may have been introduced together with their host plants.

Introduction

Protists tend to have wider distributions and a lower degree of endemism than multicellular organisms. Their huge population sizes and efficient dispersion over large areas, facilitated by their small size (Finlay, 2002), would be the primary causes of these phenomena. The underlying question is to what extent the current geographic barriers and historical geologic events have restrained the dispersion of protists, and if their influence can be traced in the distribution of the organisms that exist today. Some authors (Fenchel & Finlay, 2004) defend the "everything is everywhere" hypothesis, which states that protist species present in a given location would be a function of only their habitat properties and not of restricted dispersion. However, others (e.g. Smith & Wilkinson, 2007; Vanormelingen et al, 2008) have found evidence in favour of the "moderate endemicity" hypothesis that at least some protists have geographically restricted distributions (Foissner, 1999, 2006; Foissner et al, 2008).

Both hypotheses were originally proposed on the basis of morphological evidence and known occurrences, causing the debate to be blurred by possible misidentifications and under-sampling artifacts (Mitchell & Meisterfeld, 2005). Later, the advent of phylogeographic

methods based on molecular data has improved the resolution for detecting and analysing variability between populations and searching for recent dispersal events. These methods have also shed light on the existence of cryptic species complexes (Amato et al., 2007; Smirnov, 2007; Morard et al., 2009; Douglas et al., 2011) sharing a common morphology but genetically distinct, which contribute to create an even more complex scenario. Clear molecular evidence of geographically restricted 18S rDNA sequence types (ribotypes) has been found in foraminifera (Darling et al. 2007; Aurahs et al. 2009), diatoms (Evans et al 2009; Sorhannus et al. 2010) and Cercozoa (Bass et al. 2007). Here we provide a striking example of such geographical genetic differentiation in Amoebozoa, using myxomycetes which lend themselves especially well to such studies as DNA can be extracted and sequenced from fruiting bodies already well preserved in herbaria from many globally widespread locations.

Myxomycetes (also called plasmodial slime moulds or myxogastrids) are a group of eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems (Stephenson & Landolt 2009) that are now classified in the protozoan phylum Amoebozoa (Cavalier-Smith et al. 2004; Smirnov et al. 2011). We are still far from fully understanding what are the mechanisms that operate on their individual distributions and their general biogeographic patterns. Attending to available data, many myxomycetes seem to be cosmopolitan (Martin & Alexopoulos, 1969), but there are also some examples of species with a more or less restricted distribution (Stephenson et al, 2008).

The present paper focuses on the myxomycete *Badhamia melanospora* Speg, a species usually found on decaying Cactaceae and other succulent plants. Its fruiting bodies (sporocarps) are easily visible, since they form groups of whitish-grey little balls, approximately 1 mm in diam., which contain dark coloured, warted, reticulate spores. The morphology of *B. melanospora* is highly variable with presence or absence of stalked sporocarps, and differences in shape, ornamentation and size of the spores; but it was not previously known if this morphological variation is an expression of phenotypic plasticity or phenotypic evidence of actual genetic divergence. *B. melanospora* appears to be mostly restricted to the arid regions of America, where it is very frequent. It has never been collected in the Asian arid regions (Novozhilov et al, 2009), and only rarely in other parts of the world, including intensively studied regions like Europe, where it is most frequently found growing on introduced cactae (GBIF, www.gbif.org, last accessed February 2011). In localities outside America, it is most frequently found growing on introduced cacti.

This study reports an analysis of intraspecific DNA sequence variation in *B. melanospora* on the entire known geographical range of the species, using a fragment of the small subunit ribosomal DNA (SSU rDNA). Genetic data has been compared with the main character distinguishing clades, i.e. the spore morphology, using scanning electron microscopy (SEM). The geographical distribution of the variants is also explored for better understanding of the evolutionary history of the species. Clade-specific adaptations to locally different environmental conditions have been analyzed by comparing niche models.

Methods

Sampling

A preliminary map of the known distribution area of *B. melanospora* was generated using data available in GBIF Data Portal (www.gbif.org, last accessed February 2011). The map was completed with more collections made by several myxomycetologists - S. L. Stephenson, M. Meyer, L. H. Cavalcanti, and R. McHugh. Specimens comprising more than 10 sporophores were selected for DNA extraction, resulting in a total of 125 herbarium specimens from 91 different localities (Supplementary Table S1) from North America (Mexico and USA), South America (Brazil, Argentina, Chile), Europe (Spain, France), North Africa (Morocco), Madagascar, and Atlantic islands (Canary Islands, Ascension island) selected for this study. All collections were represented by material that fruited in the field under natural conditions.

DNA extraction and sequencing

DNA was extracted as described elsewhere (Fiore-Donno et al., 2008). The largest fragment of the SSU rDNA (533bp) that is free of type I intron insertion sites and displays sufficient variability was amplified to assess the genetic structure of populations of *B. melanospora*. With the use of specific primers it was easy to sequence, allowing us to maximize the number of specimens processed, and to make a large scale study. It was amplified by polymerase chain reaction (PCR) using the primers SA' (TGGTTGATCCTGCCAGTAGTGT) and SU19R (TGTCCTCTAATTGTTACT CGA), Mangotaq mix (Bioline) and the following cycling parameters: 45 s initial denaturation at 94ºC followed by 33 cycles of 25 s denaturation at 94ºC, 60 s annealing at 42ºC, and polymerization at 72 ºC for 3.5 min. We also obtained nearly complete SSU rRNA gene sequences (1651 bp) from seven specimens of *B. melanospora*, and from six other species of the genera *Badhamia* and *Physarum*. Four overlapping sequence frangments were obtained using the primers SA', SU19R, and S4, S900R, S11.5 SR15, DA2, RB2 (Fiore-Donno et al., 2008) and same PCR conditions. The internal transcribed spacer (ITS) gene was also initially amplified, but it showed an enormous sequence and length variation and was discarded. Purified PCR products (PureClean kit, Ecogen) were sequenced directly by Macrogen Korea. All new sequences were submitted to GenBank.

Phylogenetic analyses

Sequences were automatically aligned and characterized with Geneious 5.4 (Drummond et al., 2011) and the obtained alignment was corrected by hand using Bioedit 7.0.9.0 (Hall, 1999). A prospective phylogenetic analysis with all available nearly complete SSU rDNA sequences from *Badhamia* and *Physarum* was made, and the species recovered as the more closely related to *B. melanospora* (data not shown) were selected as outgroups for subsequent analyses. Then, the best available model of molecular evolution was selected for the 533bp SSU rDNA fragment with MrModeltest 2.3 (Posada & Crandall, 1998; Nylander, 2004), and GTR + Γ + I was the best-fit. Phylogenetic trees were primarily constructed using Bayesian inference (BI), with MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Two identical searches with ten million generations each (chain temperature = 0.2; sample frequency = 1000) were performed. In both runs, probabilities converged on the same stable value approximately after generation 8,000,000. A 50% majority-rule consensus tree was calculated, and posterior probability (PP) was used as an estimate of

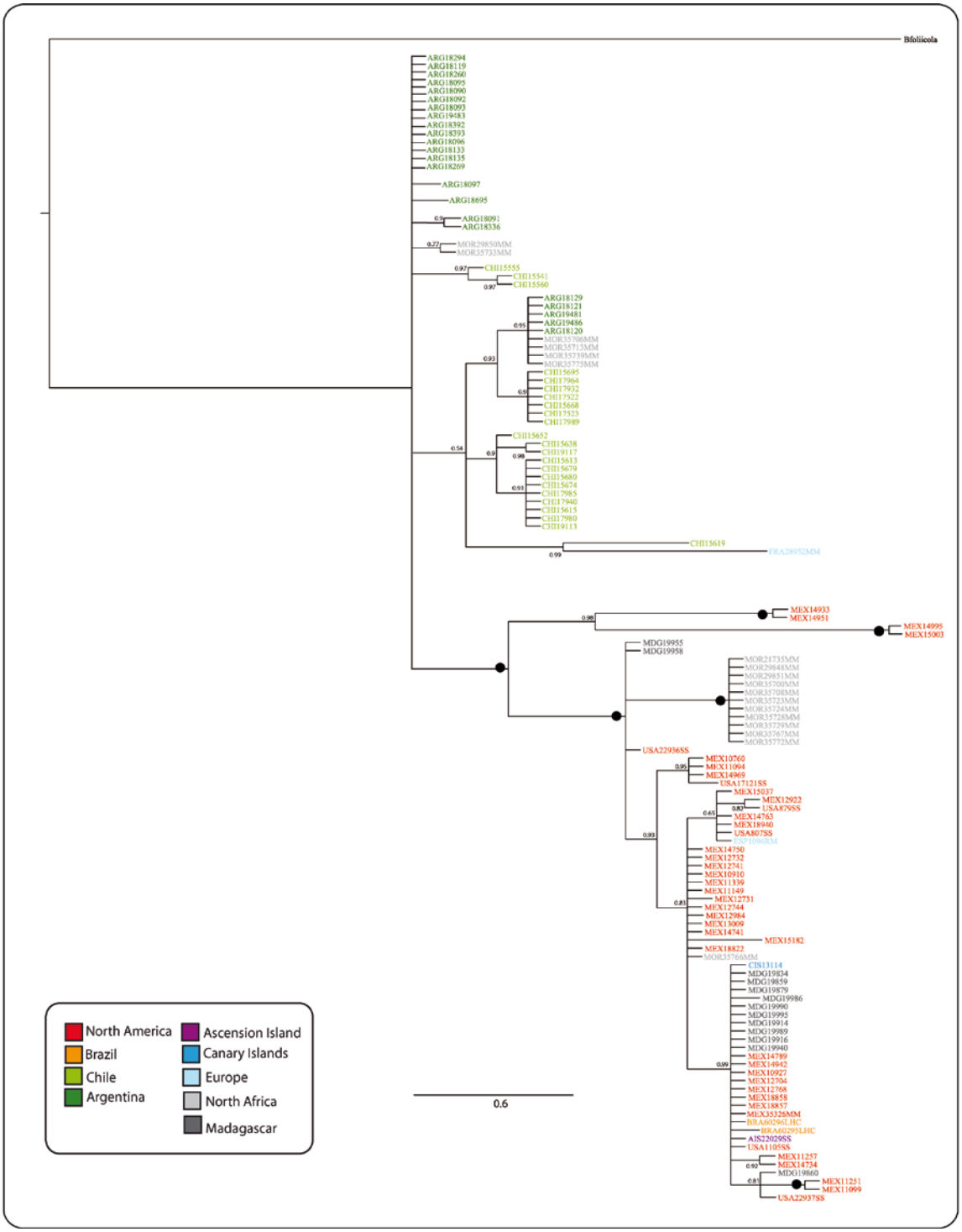

Figure 1 – Fifty percent majority-rule rooted consensus tree of a 533bp fragment of the small subunit rDNA (SSU) of 125 samples of *Badhamia melanospora* and *B. foliicola* as outgroup obtained by Bayesian inference. Colored branches indicate the origin of the samples. The scale bar represents evolutionary distance in changes per site. Posterior probabilities (PP) are presented in each node, black circles represent PP = 1.

robustness. All BI analyses were carried out on the freely available Bioportal (www.bioportal.uio.no). Parameter estimates were graphically analyzed to assess stability (Tracer ver. 1.0.1, Rambaut and Drummond, 2003).

Ribotype networks, representing unique DNA sequences separated by mutational steps, were constructed using statistical parsimony with TCS software (http://darwin.uvigo.es/software/tcs.html).

Scanning Electron Microscopy

Scanning electron microscopy (SEM) images were obtained after critical-point drying of 31 specimens distributed across the whole phylogeny. SEM analyses and photomicrographs were made by the Service of Scanning Electron Microscopy of the Royal Botanic Garden of Madrid, employing a Jeol T 330 A scanning electron microscope, at 10-15 kV. The largest diameter of the spores was measured in 10 spores per specimen.

Niche models

To predict species' occurrence over geographic space, we used a maximum entropy model implemented in the program Maxent version 3.3.3a, July 2010 (Phillips et al, 2006; Phillips & Dudik, 2008), in which the probability of a species' occurrence is estimated based on a uniform probability distribution (maximum entropy) and on presence data provided by the user. Maxent was chosen because of its good performance using presence-only data (Elith et al, 2006; Graham & Hijmans, 2006), and because it is a powerful tool in comparison with other methods (Elith et al, 2006) even in the presence of small datasets (Hernández et al, 2006). Separate niche models were generated for each of the main groups of

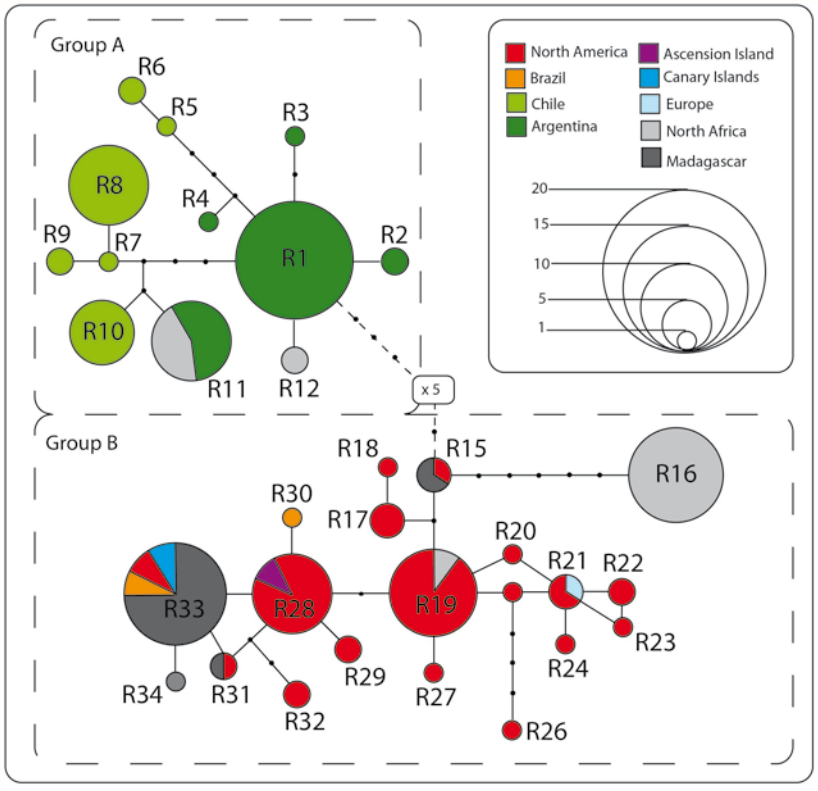

Figure 2 – Ribotype network of a 533bp fragment of the small subunit rDNA (SSU) of of 123 samples of *Badhamia melanospora*. Circle size is proportional to the number of samples within a given ribotype (see Supplementary Material S2), and dots between ribotypes represent unobserved, inferred ribotypes. Lines between ribotypes represent mutational steps between alleles. Colors denote sample origin.

ribotypes, and were calculated with Maxent using Bioclim variables from WorldClim (Hijmans et al, 2005) (www.worldclim.org) with a 2.5 arc-minutes resolution. Only collections that were precisely geo-referenced were used to generate the models (84 localities). The locality of the specimen 35767MM from Morocco (coordinates -10.022778, 30.60547) was excluded from the analyses because it was not covered by some environmental layers. Models were evaluated based on receiver operating characteristic (ROC) analysis, which generates the AUC (area under the curve) score. Outputs were compared using ENMtools (Warren et al, 2010), with D (Schoener, 1968) and I (Warren et al. 2008) as measures of niche overlap. For that, we generated 100 pseudo-replicated maxent models by random sampling from all data points, pooled for both ribotype groups. Eventually the niche overlap measures obtained from the original data were compared to the distribution of data generated by the pseudo-replicates, and differences were evaluated for their statistical significance.

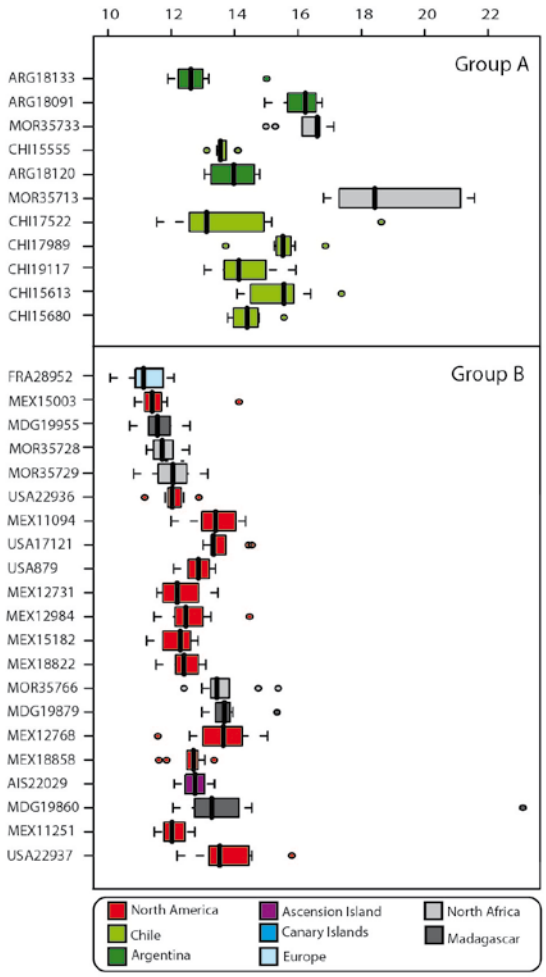

Figure 3 – Box-and-whiskers plot of the maximum diameter of spores in µm, that was measured in 10 spores per sample using SEM. Colors denote sample origin.

Results

Bayesian inference (BI)

This short gene region showed enough variability at the intraspecfic level: 87 (16.3%) variable sites, with an average percent identity of 97.2% and an average 53.3% GC-content. Most mutations were single nucleotide substitutions. The evolutionary interrelationships among all 37 *B. melanospora ribotypes* (Supplementary Material S2) found in the 125 specimens sequenced are shown in Fig. 1. One big group of 54 sequences (group A) is formed basically by specimens from South America (Chile and Argentina), but also contains six sequences from Morocco and one from France. A well supported, divergent clade (B) groups together all North American collections (mainly Mexico; six from USA) together with two from Brazil and all but one of the specimens from the old world (Morocco, Canary Islands, Ascension Island, Spain, Madagascar). The putatively more ancestral nodes of the tree are not well resolved, but most basal clades are Argentinian populations.

Phylogenetic network estimation using statistical parsimony (TCS)

TCS analyses of the sequences (Fig.

2) were highly congruent with the results yielded by BI. Some highly divergent ribotypes, R13 (CHI15619), R14 (FRA28952), R35 (MEX14933), R36 (MEX14951), and R37 (MEX14995, MEX15003) did not join the network, and were excluded from the final analysis. The remaining sequences formed two well-delimited groups of ribotypes. The first group, ribotype group A, has 12 different ribotypes, mostly from South American populations - Argentina and Chile, but there are also some from Morocco. It is remarkable that sequences from Chilean populations are divided and located in two different parts of the network, but none of them was identical to any Argentinian sequence, so no ribotypes are shared by populations on both sides of the Andes. One of the two different Moroccan ribotypes is shared with Argentinian populations; the other is closely related to the most common Argentinian ribotype.

The second group, ribotype group B has 20 ribotypes, sequenced from specimens from North America - USA and Mexico, Brazil and nearly all collections from the Old World - North Africa, Madagascar, Europe, and Atlantic oceanic islands – Canary Islands, Ascension Island, and Brazil. A group of closely related ribotypes is formed by sequences from Brazil, the Canary Islands, Madagascar, and Ascension Island. There is also a divergent, abundant Moroccan ribotype. Other ribotypes from Europe, Morocco, and Madagascar appear associated to other parts of the network, in most cases being closely related to North American populations.

Scanning electron microscopy (SEM)

Slight differences in the size of the spores and in the ornamentation of the walls, have been detected between ribotype group A and ribotype group B in SEM. Specimens from ribotype group A have a higher spore size range (Fig. 3), and a much more variable wall ornamentation (Fig. 4), sometimes showing a very marked reticulum and a polygonal shape. Spores from specimens from ribotype group B have a more uniform size range (Fig. 3), and ornamentation (Fig. 4), with a generally less marked reticulum and round in shape.

Environmental niche models

The geographic structure found in the American ribotypes could have been mainly caused by limited dispersion, or alternatively by an efficient dispersion but different environmental preferences of each ribotype group. To test this, environmental niche models were made separately with occurrences from each group of ribotypes, and they were compared both visually and statistically. The results show differences in ecological preferences of the two ribotype groups. In both cases (Fig. 5, Fig. 6), predicted areas are located in warm arid territories. Deserts from Eurasia are not predicted in any of the models. In the case of ribotype group A, the map obtained predicts presences in more restricted areas, situated near the western coasts of the continents. Ribotype group B, despite being genetically less diverse, has a broader niche and its high probability areas are mainly in intra-continental localities.

The identity of the environmental niche models was numerically compared using D and I indices from ENMTools. The values of D and I obtained from the original data (0.2705 and 0.5257, respectively) were significantly smaller than the average values of the distribution obtained from the random pseudo-replicated models (Fig. 7). In conclusion, the environmental niche models of the ribotype groups are less similar than random, so there are

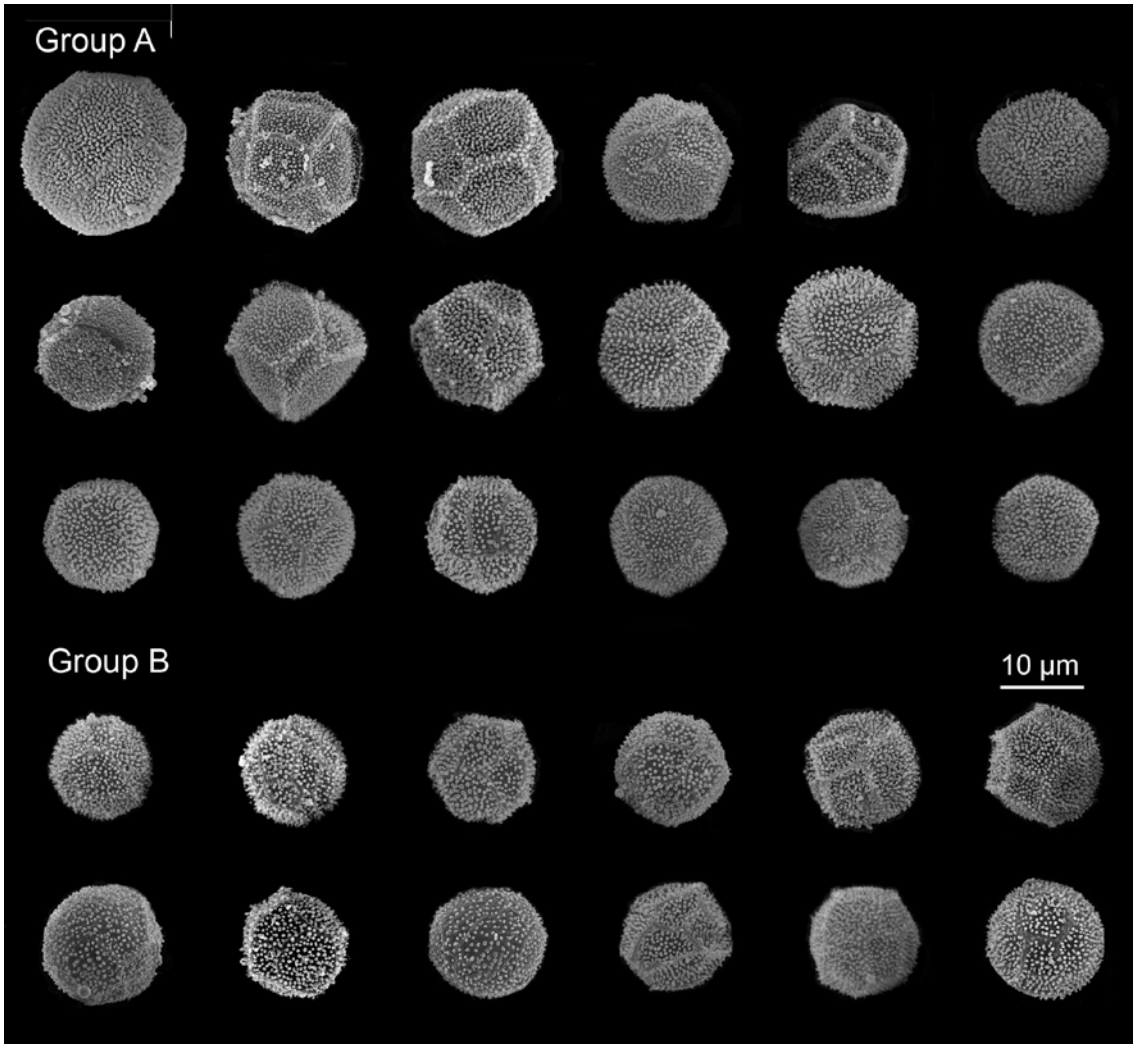

Figure 4 – SEM pictures of spores from specimens from ribotype groups A and B.

differences in the ecological preferences of the ribotype groups, group B having a wider niche than group A.

Discussion

This work represents a first step in the study of infraspecific variation of myxomycetes in a geographical context. Our data reveal a cryptic diversity in an otherwise rather morphologically uniform complex of species, and shed light on many important unresolved questions about their ecological preferences.

Geographic Differentiation Amongst Ribotypes

Our data provide a biogeographical signal that can be interpreted to understand the history of the species. If our tree is correctly rooted, then as most basal clades in the BI tree are Argentinian populations, it would appear that *Badhamia melanospora* has its most probable origin in South America. *B. melanospora* could have moved to North America by a non-recent single long distance colonization event, because all North American specimens are part of a well supported divergent clade

that includes most collections from the Old World but does not include any sequence from Argentina or Chile. The TCS analysis shows similar results with most closely related sequences from ribotype group A and ribotype group B separated by 9 missing ribotypes. However, as our tree is rooted by a single genetically very distant outgroup, there is a possibility that the position of the root within *B. melanospora* is slightly misplaced. If its root were actually between group A and clade B, as we suspect could be true, not within group A as shown on Fig. 1, then group A would also be a clade and not actually basal and ancestral. In that case an alternative scenario of the ancestor being widespread throughout America and simply diverging allopatrically would be more plausible.

However our data do imply some long distance migration. They suggest quite strongly that North American populations were the main source for populations from the Old World, except for two groups of North African collections and a highly divergent French specimen that were more closely related to South American populations.

Another interesting result is that none of the ribotypes were found at both sides of the Andes. The topology of the tree and the TCS network do not show, however, two clearly defined groups of populations from one side and the other. The most likely explanation is that the Andes acted as a semi-permeable barrier allowing multiple colonization events across the mountain range.

The morphological differences we found between ribotype groups support these conclusions. The average diameter of the spores is different in both groups, but they have overlapping size ranges. Also, the spore ornamentation was usually different, but in both groups it is possible to find specimens that could be considered as belonging to either group. Thus, the morphological characters studied are not useful as diagnostic characters, but show that genetic differentiation between the groups has had some consequences for morphology.

Our results are consistent with data from mating experiments in cultures of different species of myxomycetes (El Hage et al., 2000; Clark, 2000; Clark & Stephenson, 2000; Irawan et al, 2000), which found that at least some myxomycete species are not genetically ubiquitous. All these data showed that a number of common and widespread morphospecies may actually consist of complexes of apomictic clonal lines, that can be geographically restricted in some cases (Clark, 2004). These lines would be reproductively isolated, so they could accumulate mutations over time and evolve independently. After a long period of isolation they would present minor morphological differences, reflecting specific adaptations to the particular set of environmental conditions in which they occur (Stephenson et al, 2008).

Also, a phylogeny of ITS sequences of 14 specimens of *Didymium squamulosum* (Winsett & Stephenson, 2008) showed variation among geographically separated isolates, but in that case it was not possible to extract any biogeographical conclusions. More recently, Fiore-Donno et al. (2011) studied two closely related species of *Lamproderma*. In that case, the distinct genotypes showed morphological differences, but genetic patterns were not related to the geographical origin of the specimens.

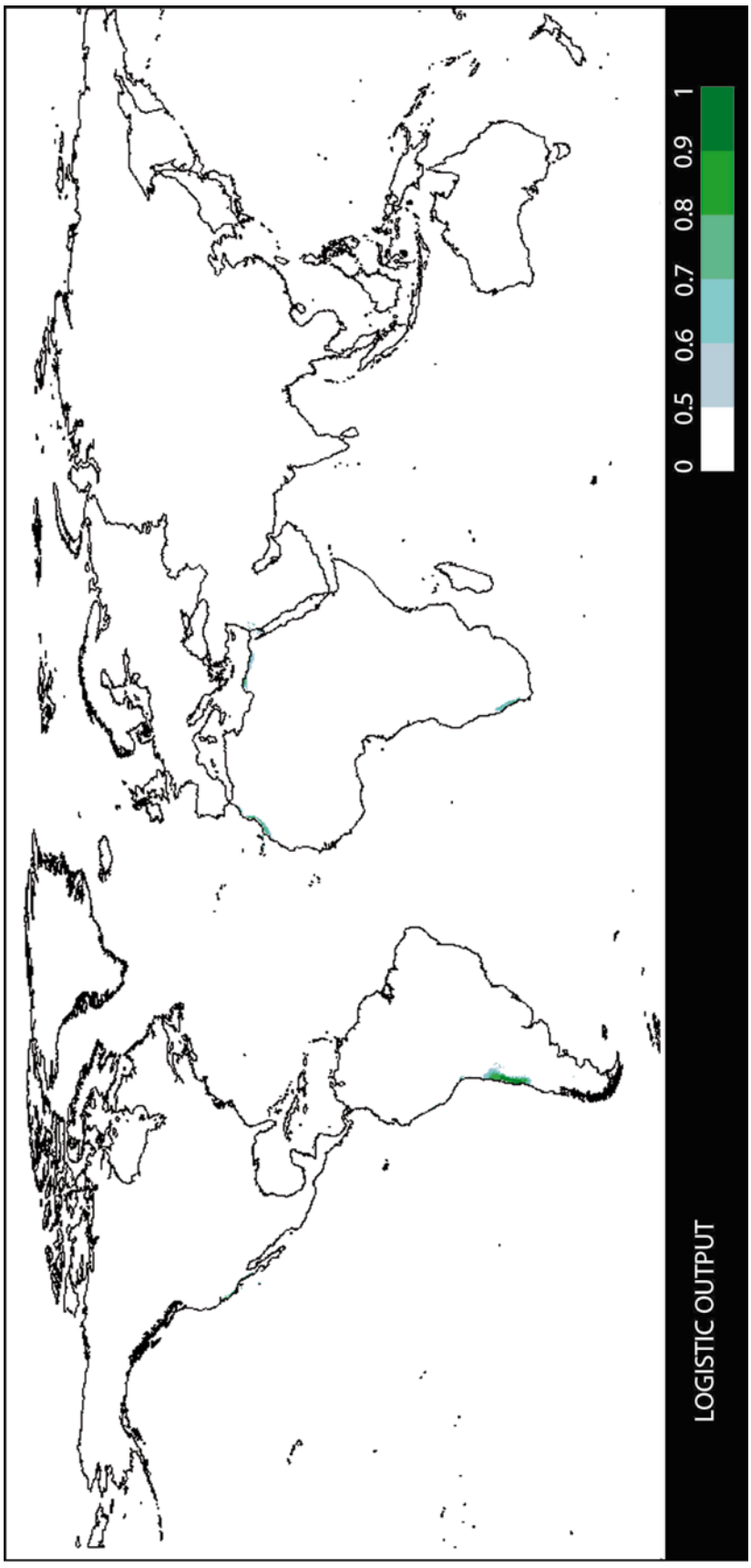

Figure 5 – Predictive ecological models based on the Maxent algorithm of ribotype group A. Probabilities of presence >0.5 are represented using different colour shades.

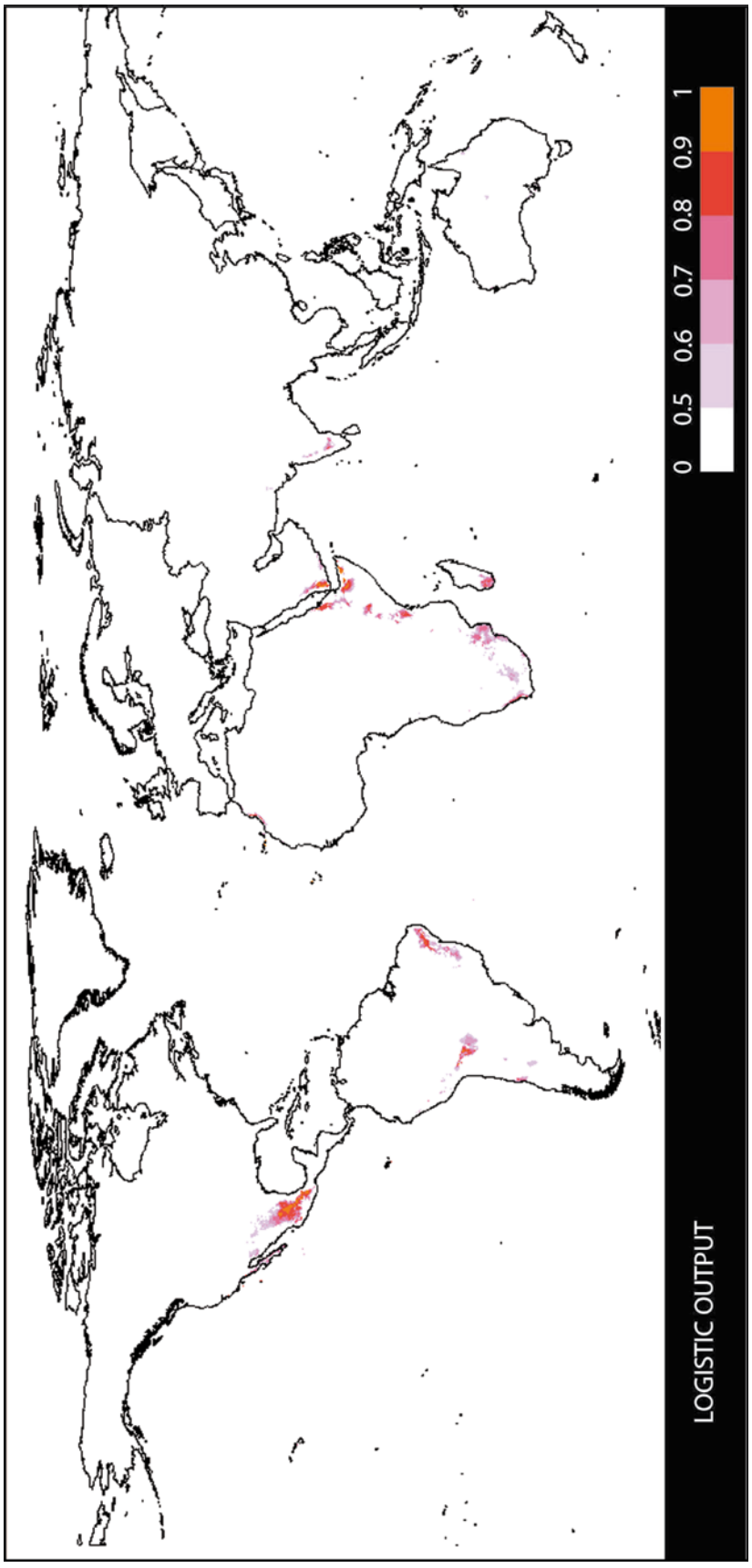

Figure 6 – Predictive ecological models based on the Maxent algorithm of ribotype group B. Probabilities of presence >0.5 are represented using different colour shades.

Ribotype groups have different environmental niches

Environmental niche models for the detected ribotype groups were calculated using climatic variables, to infer the probability of presence of the species on a large scale, and provide results that can be projected as maps and subsequently compared. Microhabitat variables may also be very important for the species survival, but probably with stronger predictive effects at a local scale than at a global scale (Aguilar & Lado, unpublished). The models provide evidence for differences in ecological preferences of the ribotype groups. However, these differences could have appeared before or after isolation by distance. One possibility is that, pre-existing differences in ecological tolerance may have led to a greater success of certain lineages in some areas. However, the fact that one ribotype from group B was found only once in Spain and many times in Mexico and that another was found once in the Canary Islands and once in Ascension Island but many times in Mexico, shows that the North American ribotypes can survive in Europe or ecologically disparate Altantic islands, strongly suggesting that the absence of most of them from these regions is because of weak dispersal not ecological unsuitability.

Ribotype group B, despite being genetically less diverse, has a bigger area predicted as ecologically suitable by its model. Therefore, in the case of *B. melanospora*, genetic diversity may not be related to niche breadth and does not imply a greater colonization ability. It is important to note that several areas in the world without any information about the presence of *B. melanospora* have a very high probability prediction in the models. This is so for Yemen and Somalia, the coast of Libya and Egypt, and South Africa. Most of these areas have never been surveyed for myxomycetes, and this lack of data makes it harder to interpret our results, as we do not know whether *B. melanospora* is present or absent from them. It would be worthwhile searching for it in these areas; if it were not found, this would strengthen the conclusion that *B. melanospora* has been able to reach all ecologically suitable areas during its dispersive processes.

Human introduction

Although American populations have a strong structure, it is striking that old world populations appear scattered throughout the tree, but in most cases belonging within a group of closely related ribotypes in group B. This pattern is most simply explained by multiple colonization events from the Americas to Europe, oceanic islands, Africa and Madagascar. In all cases the old world strains are nested with the ancestrally new world groups A and B. The four ribotypes found in Morocco do not cluster together; one is almost identical to Mexican sequences and one to Argentinian sequences, whereas two are distinct Moroccan ribotypes. We suggest there were four distant colonizations of Morocco from North America, two so recent as to be almost identical to their source strains and two more ancient – long enough ago for separate ribotypes to have evolved after colonization. We postulate three separate colonizations of Madagascar, all most likely from Mexico.

Indeed, most specimens collected out of America were found on plants originally introduced from North or Central America (*Opuntia*, *Agave*), which quite strongly supports the hypothesis that the dispersion of *B. melanospora* has been facilitated by human introductions of American succulent

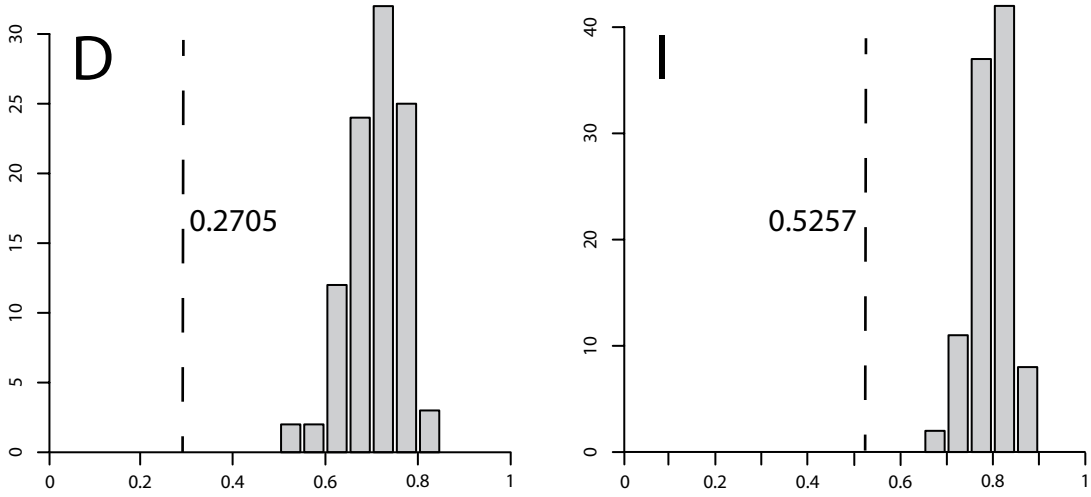

Figure 8 – Niche comprarisons of ribotype groups A and B based on D and I as measures of the niche overlap. Dotted lines represent niche overlap measures of the original data, and bars show the expected degree of niche overlap when samples are drawn from the same distribution (i.e., pooled samples of occurrence points from the ribotype groups).

plants into the Old World. If these strains had evolved in situ one would expect them to be on native old world plants. The two Brazilian sequences are also most likely the result of a single introduction from Mexico and are of the same ribotype that was apparently introduced into Ascension Island and the Canaries. If our interpretations are correct, then *Badhamia melanospora* provides the first documented human introduction of a myxomycete. Given the close similarity of most putatively introduced strains it is unlikely that our results are misled by the absence of data from Africa due to an incomplete sampling. Nonetheless search for *B. melanospora* in southern and East Africa is an important test of our human introduction hypothesis.

Badhamia melanospora is probably a cryptospecies complex

Due in part to their small size and lack of morphological characters, two or more protist species can have the same or very similar morphologies, forming a biological species complex. If species complexes are not appropriately detected and characterised, and are treated as single species, results of many different studies will be affected: species richness in a habitat may be underestimated, biogeographical inferences may be invalid, and species' ecological tolerance and habitat range could be overestimated. Therefore, it is a pivotal challenge in systematics and evolutionary ecology to recognise cryptic species, in order to describe and understand biodiversity. Recent application of molecular markers across a wide range of protist taxa has shown that cryptic species complexes are indeed much more common than has been traditionally assumed (Amato et al., 2007; Bass et al. 2009; Douglas et al., 2011; Howe et al. 2009; Poulíčková et al. 2010; Morard et al., 2009; Smirnov, 2007).

For making reasonable assertions about whether a particular myxomycete clade represents a cryptic species, it would be necessary to evaluate if these organisms are reproductively isolated, and/or if they are separated from other strains by a relatively long branch. In the case of *B. melanospora*, group B constitutes a well defined clade, with high support, and separated from other sequences by a relatively long branch. This makes it likely that it is reproductively isolated from group A and forms an independent evolutionary line. In addition, the ribotype groups described here have different geographical distributions, different ecological preferences, and a slightly different morphology. Thus groups A and B are genetically, morphologically and geographically strongly differentiated and it is unlikely that there is much, if any gene flow between them. Very likely they are distinct biological species each of highly restricted geographic distribution. None of the morphological characters studied allow an a priori classification of the specimens in either group, and therefore they cannot be considered as diagnostic characters. It would not be surprising if the two groups each include several cryptic biological species.

Conclusions

In summary, *B. melanospora* is a complex case in which limited dispersion, isolation by distance, host specificity and other ecological parameters have acted, giving rise to a set of at least two cryptospecies with slight but not completely distinguishable morphologies. In addition, human introduction of host plants may have played an important role in facilitating multiple long distance colonization events from the Americas to the Old World, as well as one putative case from Mexico to Brazil. Myxomycete (myxogastrid) Amoebozoa are among the most widely distributed of all terrestrial organisms, but also amongst the least known. Owing to a serious lack of data about species distribution, ecological, phylogeographic, and biogeographic studies of myxogastrids is an immense challenge; species boundaries and biogeography need to be much better defined to provide a proper basis for more reliable studies of their diversity, ecology and systematics.

Aknowledgements

The authors would like to acknowledge S. L Stephenson, M. Meyer, L. H. Cavalcanti, R. McHugh for contributing with specimens and providing comments on the species' distribution and morphology. Fátima Durán, Guillermo Sanjuanbenito, Gemma Andreu, Yolanda Ruiz, and Juan Carlos Hernández for technician work. This work has been supported by the Research Projects CGL2005-00320/BOS and CGL2008-00720/BOS of the Ministry of Science and Innovation of Spain.

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Capítulo 6: Descripción de una Nueva Especie

La descripción de nuevas morfoespecies es de gran importancia, pues permite ampliar el conocimiento de la biodiversidad de estos grupos, y ayuda a reducir el déficit linneano que, como se ha explicado en la introducción general, también afecta a numerosas otras cuestiones, entre ellas el conocimiento de los patrones generales ecológicos y biogeográficos de estos organismos. Existe todavía una gran cantidad de biodiversidad que nos es totalmente desconocida, y que puede llegar a extinguirse antes de que sea conocida y descrita. Este desconocimiento se encuentra además fuertemente sesgado hacia determinados tipos de organismos y hacia determinados tipos de ambientes (Mora et al, 2011). En el caso de los mixomicetes el ambiente desértico ha comenzado a estudiarse muy recientemente. Este hábitat, que parecería a priori ser poco apto para la supervivencia de estos organismos, sin embargo está mostrando ser un ambiente al que muchos mixomicetes hasta ahora desconocidos están específicamente adaptados.

En este capítulo se incluye la descripción de una nueva especie de *Perichaena* encontrada en desiertos Americanos que ha sido publicada en el siguente artículo:

Lado C, Wrigley de Basanta D, Estrada-Torres A, García Carvajal E, Aguilar M, Hernández-Crespo JC. (2009). Description of a new species of *Perichaena* (Myxomycetes) from arid areas of Argentina. Anales Jard. Bot. Madrid 66S1:63-70.

Resumen: Se describe una nueva especie, *Perichaena calongei,* que fue encontrada en el desierto de Monte, en las zonas áridas del noroeste de Argentina. Los cuerpos fructíferos se encontraron fructificados en el campo, también se obtuvieron por cultivo en cámara húmeda de plantas recolectadas en las provincias de Catamarca, Jujuy, La Rioja, Salta y San Juan. La combinación de caracteres de la morfología del esporocarpo, de la estructura y tipo de dehiscencia del peridio, y de la ornamentación del capilicio, distinguen esta especie del resto de las conocidas en el género. La morfología de la especie se analizó con un microscopio óptico dotado de contraste interferencial de Nomarski y con un microscopio electrónico de barrido, se incluyen ilustraciones de las estructuras observadas. Se propone una clave dicotómica para la identificación, a nivel mundial, de las especies estipitadas del género *Perichaena.*

Anales del Jardín Botánico de Madrid
Vol. 66S1: 63-70, 2009
ISSN: 0211-1322
doi: 10.3989/ajbm.2229

Description of a new species of *Perichaena* (Myxomycetes) from arid areas of Argentina

by

[1] Carlos Lado, [1] Diana Wrigley de Basanta, [2] Arturo Estrada-Torres, [1] Eva García Carvajal, [1] María Aguilar & [1] Juan Carlos Hernández-Crespo

[1] Real Jardín Botánico, CSIC, Plaza de Murillo 2, E-28014 Madrid, Spain. lado@rjb.csic.es
[2] Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, km 10,5 carretera Texmelucan-Tlaxcala, Ixtacuixtla, 90122, Tlaxcala, México. arturomixo@hotmail.com

Abstract

Lado, C., Wrigley de Basanta, D., Estrada-Torres, A., García Carvajal, E., Aguilar, M. & Hernández-Crespo, J.C. 2009. Description of a new species of Perichaena (Myxomycetes) from arid areas of Argentina. *Anales Jard. Bot. Madrid* 66S1: 63-70.

A new species of the myxomycete genus *Perichaena* is described in this paper. The new species, named *Perichaena calongei*, was found during intensive studies of arid areas of the Monte desert in Argentina. It has been found directly in the field from the provinces of Catamarca, La Rioja, Salta and San Juan, in the Northwest of Argentina, and isolated from moist chamber cultures of native plant species collected in Catamarca, Jujuy, Salta and San Juan. The characters that make this species unique in the genus are the combination of the morphology of the sporocarps, the structure and dehiscence of the peridium, and the ornamentation of the capillitium. The morphology of the myxomycete specimens was examined using light microscopy with Nomarski interference contrast, and scanning electron microscopy. Micrographs of relevant morphological characters are included. A key to facilitate the identification of the stipitate species of *Perichaena* is also proposed.

Keywords: Monte desert, morphogenesis, Mycetozoa, Protista, *Puya,* SEM, slime mould, taxonomy.

Resumen

Lado, C., Wrigley de Basanta, D., Estrada-Torres, A., García Carvajal, E., Aguilar, M. & Hernández-Crespo, J.C. 2009. Descripción de una nueva especie de Perichaena (Myxomycetes) encontrada en zonas áridas de Argentina. *Anales Jard. Bot. Madrid* 66S1: 63-70 (en inglés).

Se describe una nueva especie, *Perichaena calongei,* que fue encontrada en el desierto de Monte, en las zonas áridas del noroeste de Argentina. Los cuerpos fructíferos se encontraron fructificados en el campo, también se obtuvieron por cultivo en cámara húmeda de plantas recolectadas en las provincias de Catamarca, Jujuy, La Rioja, Salta y San Juan. La combinación de caracteres de la morfología del esporocarpo, de la estructura y tipo de dehiscencia del peridio, y de la ornamentación del capilicio, distinguen esta especie del resto de las conocidas en el género. La morfología de la especie se analizó con un microscopio óptico dotado de contraste interferencial de Nomarski y con un microscopio electrónico de barrido, se incluyen ilustraciones de las estructuras observadas. Se propone una clave dicotómica para la identificación, a nivel mundial, de las especies estipitadas del género *Perichaena.*

Palabras clave: desierto de Monte, morfogénesis, Mycetozoa, Protista, *Puya*, MEB, hongos mucilaginosos, taxonomía.

Introduction

The genus *Perichaena* (order Trichiales, Myxomycetes) was erected by Fries (1817), based on *Perichaena populina* (Alb. & Schwein) Fr., a synonym of *Perichaena corticalis* (Batsch) Rostaf. (Martin, 1966). The species of the genus *Perichaena* are mainly characterized by having simple or branched tubular capillitial threads, which are roughened, warted or spiny to minutely annulate in some species, but not marked with spiral bands (Martin & al., 1983). The capillitial threads have an irregular outline, are normally not isodiametric, and are generally perforated with pits only visible by SEM.

The last taxonomic revision of this genus was made by Keller (1971), in which he established the limits between different species, and elaborated a key of the 13

species known then. The taxonomic problems in the group, detailed by Keller in this paper, were later updated by Keller & Eliasson (1992). Some species of the genus have a wide distribution and are ubiquitous, but others have a very restricted distribution, or are known only from the type locality.

This genus includes 26 species according to Hernández-Crespo & Lado (2005) and Lado (2008). Six of them were described with stipitate sporocarps. These are *Perichaena pulcherrima* Petch (Petch, 1909); *P. pedata* (Lister & G. Lister) G. Lister ex E. Jahn (Jahn, 1919); *P. reticulospora* H.W. Keller & D.R. Reynolds (Keller & Reynolds, 1971); *P. papulosa* C.H. Liu & J.H. Chang (Liu & al., 2007); *P. polygonospora* Novozh., Zeml., Schnittler & S.L. Stephenson and *P. heterospinispora* Novozh., Zeml., Schnittler & S.L. Stephenson (Novozhilov & al., 2008). Another two species originally described as sessile, have been found with short stalks, or with a reduced base that can be interpreted as a very short stalk. These are *Perichaena chrysosperma* (Curr.) Lister (Lister, 1894), and *P. areolata* Rammeloo (Rammeloo, 1984a). In addition, Estrada-Torres & al. (2009), have described another one, *P. stipitata* Lado, Estrada & D. Wrigley, thus increasing the number of stipitate species to 9.

During intensive studies of different arid areas in the Neotropical Region, some stipitate specimens of *Perichaena* were collected both in the field, and also from moist chamber cultures of native plant material. The combination of the morphology of the sporocarps, the structure and dehiscence of the peridium, and the ornamentation of the capillitium were different from the known species in the genus. A detailed description, illustration and discussion are provided of this material, which we propose here as a new species.

Materials and methods

The collecting sites pertinent to this paper were located between 23°50'-30°10' South latitude and 65°27'-67°48' West longitude, along the eastern foothills of the Andes. The vegetation was xerophyllous scrubland, where in rocky areas, the rosette-leaved bromeliad *Puya* spp. predominated.

The studies involved the collection in the field of myxomycetes from known or suspected microhabitats, and removal of substrates for laboratory culture. This paper is based on material obtained from field collections on the dead leaves and moist chamber cultures of leaf bases, of *Puya* spp. The field collections and the substrate material for moist chamber cultures were made in five states of northwest Argentina (Catamarca, Jujuy, La Rioja, Salta and San Juan) by C. Lado, A. Estrada-Torres and D. Wrigley de Basanta. Field collections were dried and glued into herbarium boxes in situ. Material for moist chamber culture was air-dried in situ and transported in sealed paper bags. All the localities were geo-referenced using a GPS (Magellan eXplorist 600 Ver. 5.1, Datum WGS84).

The moist chamber cultures were prepared using pieces of dry *Puya* sp. leaf bases, which were placed on filter paper lining sterile 9 cm plastic Petri dishes. The cultures were prepared as described in Wrigley de Basanta & al. (2009). All fruiting bodies of the same species in one culture were regarded as being one collection. All the specimens are deposited in the herbarium MA-Fungi (sub Lado), and the private collection of Diana Wrigley de Basanta (dwb).

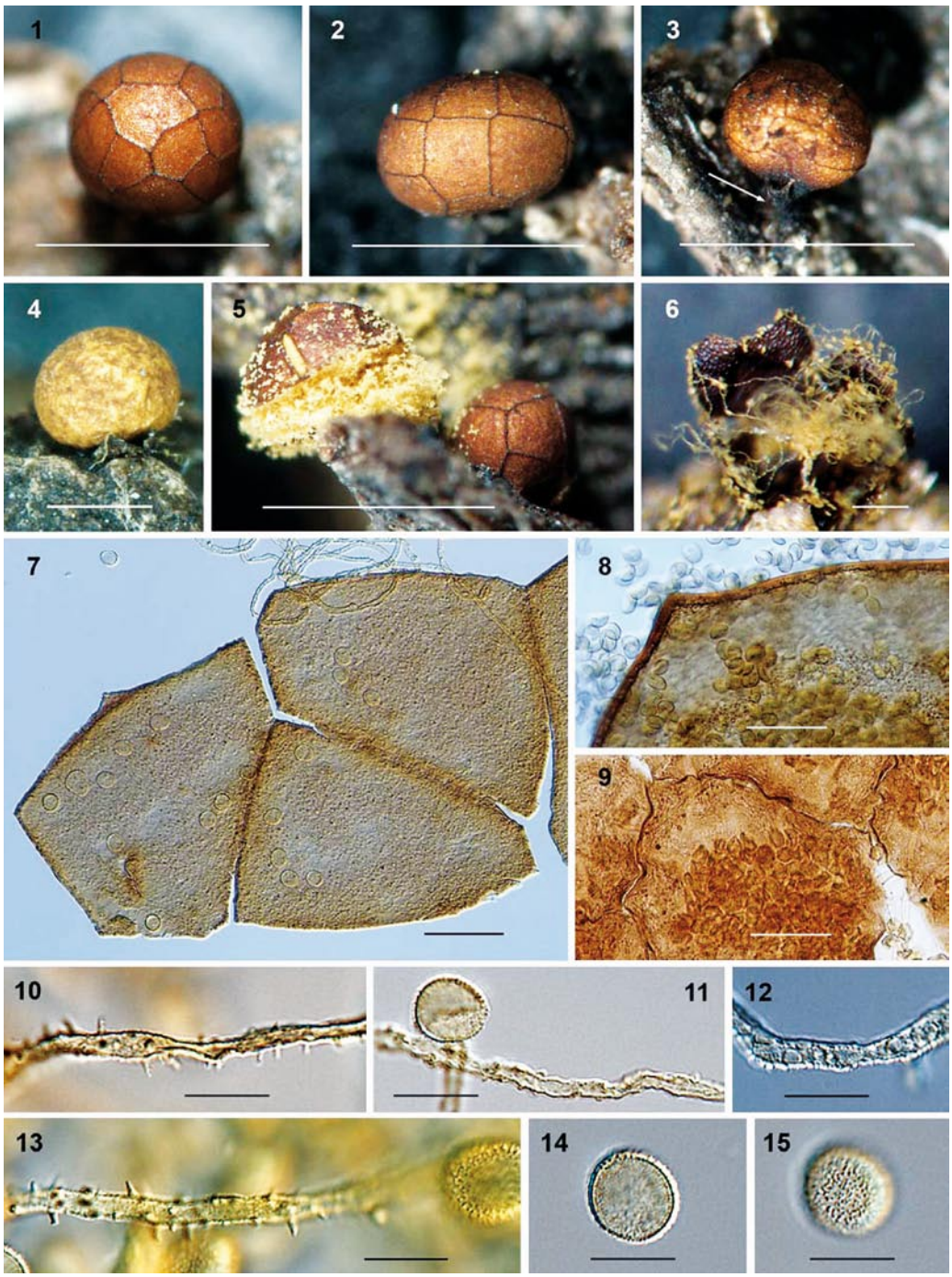
All microscope measurements and observations were made with material mounted directly in Hoyer's medium. A microscope with differential interference contrast (DIC) was used to obtain descriptive data and light micrographs. The critical-point drying technique was used for scanning electron microscopy (SEM) preparations, and the SEM analyses and photomicrographs of specimens were made by the Scanning Electron Microscopy Department of the Royal Botanic Garden of Madrid, employing a Hitachi S-3000N scanning electron microscope, at 10-15 kV. Colour notations in parentheses are from the ISCC-NBS Color-Name Charts Illustrated with Centroid Colors (Anon, 1976).

Taxonomic treatment

Perichaena calongei Lado, D. Wrigley & Estrada, **sp. nov.** Figs. 1-3, 5-22

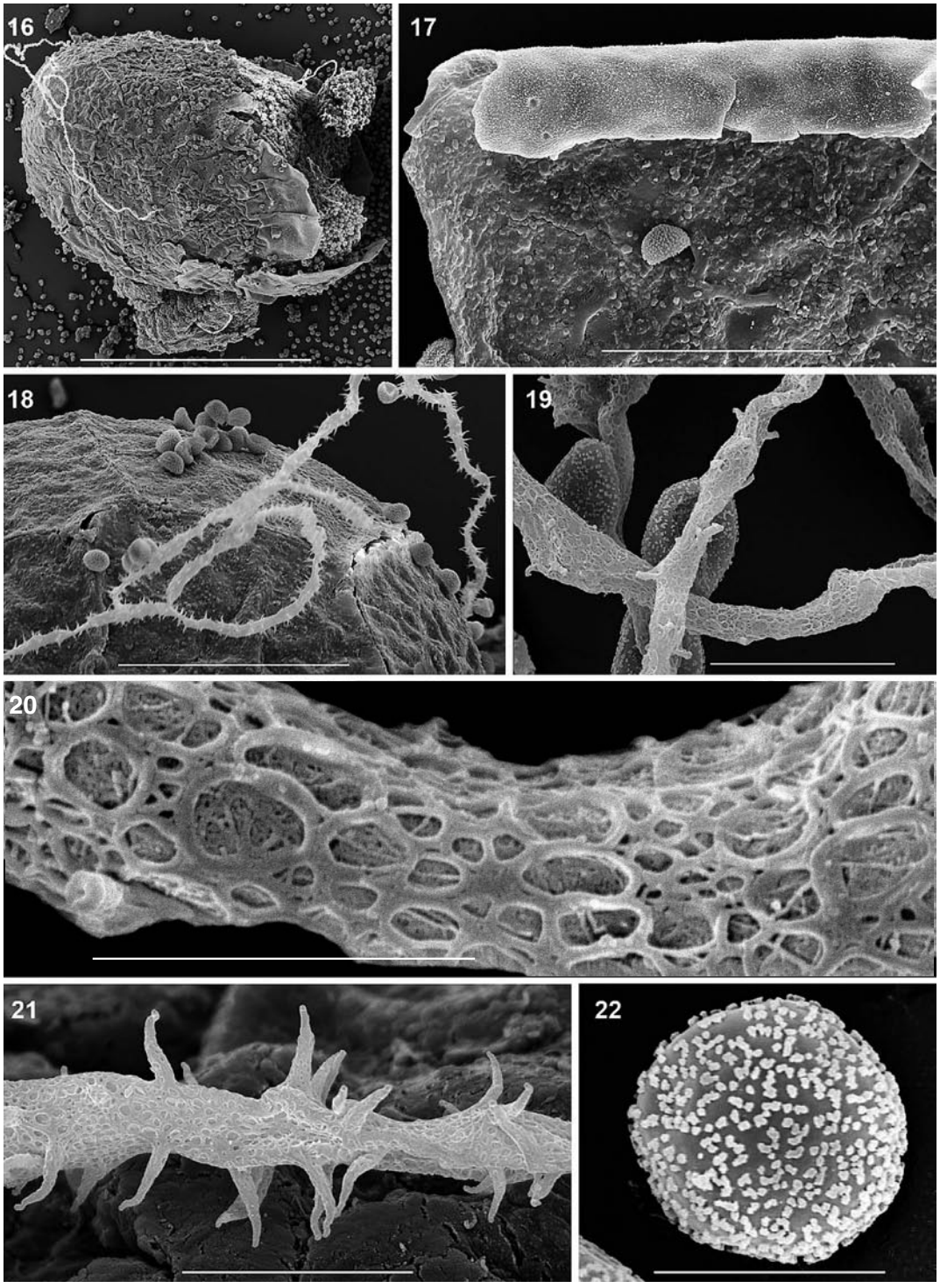
Sporocarpia dispersa, stipitata vel subsessilia. Sporotheca subglobosa, 0,2-0,8 mm *diam., flavo-aurantiaca vel fusca. Stipes cylindricus,* 0,1-0,35 mm *altus. Peridium bistratum; stratum externum coriaceum, depositum granulatae materiae includens; stratum internum membranaceum, ad externo strato valde adhaerens; in frustula polygona dehiscente. Capillitium flavum, tubulis* 2-4 µm *diam., cum ramis, non regulariter ornatum spinis, granulis vel reticulis. Sporae liberae, flavo-aurantiacae in massa, flavae luce transmissa,* 10-13,5 µm *diam., verrucosae.*

Sporophores sporocarpic, scattered or in small groups of 2-4 sporocarps, shortly stalked or subsessile. Sporotheca sub-globose, 0.2-0.8 mm diam., orange yellow (67. brill. OY - 72. d. OY) to dark brown (78. d. y Br), often with dark lines marking the edges of peridial plates (Figs. 1-3, 5). Hypothallus membranous, brownish, discoid, individual under each sporocarp. Stalk cylindrical, 0.1-0.35 mm in height, without calcium deposits, dark brown (59. d.



Figs. 1-15. 1-3, 5-15, *Perichaena calongei.* **1, 2,** Sporocarps showing peridial plates. **3,** Sporocarp showing dark stalk (arrow). **4,** *Perichaena areolata*. Sporocarp. **5,** Dehisced sporocarp showing the mass of spores and closed sporocarp. **6,** Dehisced sporocarp showing petaloid calyculus-like base. **7,** Polygonal plates of the peridium by transmitted light showing the dark borders. **8,** Detail of dark edge of peridial plate by LM. **9,** Detail of peridial plates by LM of a less mature sporocarp. **10, 13,** Capillitial tubule ornamented with spines and granules. **11,** Capillitial tubule ornamented with short spines. **12,** Capillitial tubule ornamented with a pitted reticulum. **14, 15,** Spores warted by LM. [1-3) dwb 2857 (holotype, MA-Fungi 78697); 4) GENT 10581 (typus); 5, 8, 10, 13-15) dwb 2833; 6) Lado 18242 (MA-Fungi 78680); 7, 12) Lado 18241 (MA-Fungi 78679); 9) dwb 2838; 11) Lado 18322 (MA-Fungi 78687)]. Bar: 1-3, 5 = 1 mm; 4 = 0.5 mm; 6 = 100 µm; 7-9 = 50 µm; 10-15 = 10 µm.

Anales del Jardín Botánico de Madrid 66S1: 63-70, 2009. ISSN: 0211-1322. doi: 10.3989/ajbm.2229



Figs. 16-22. *Perichaena calongei* by SEM. **16,** Whole sporocarp with short stipe. **17,** Detail of edge of a peridial plate showing the almost smooth inner surface of the inner layer. **18,** Detail of the sporocarp surface showing peridial plates. **19,** Capillitial tubules showing sections with short spines and with no spines**. 20,** Detail of a capillitial tubule showing double reticulum. **21,** Spiny section of a capillitial tubule. **22,** Spore with flattened warts. [16-22) *dwb 2857* (holotype, MA-Fungi 78697)]. Bar: 16 = 500 µm; 17 = 50 µm; 18 = 100 µm; 19 = 20 µm; 20 = 5 µm; 21, 22 = 10 µm.

Anales del Jardín Botánico de Madrid 66S1: 63-70, 2009. ISSN: 0211-1322. doi: 10.3989/ajbm.2229

Br) to blackish (Fig. 3), filled with refuse matter, with a roughened surface. Peridium double, outer layer coriaceous, with granular material, yellow (84. s. Y - 88. d. Y), to deep orange yellow (69. deep. OY - 72. d. OY) or greyish yellow (90. gy. Y) by transmitted light; inner layer membranous, yellowish, strongly adhered to the external layer, with the inner surface almost smooth by SEM (Fig. 17); dehiscing into polygonal plates (Figs. 5, 18), leaving a petaloid calyculus-like base (Fig. 6); peridial plates of 4-6 sides (Figs. 1, 7), often with a dark smooth border which is sometimes revolute (Figs. 8, 17). Columella absent. Capillitium tubular, tubules of irregular diameter, 2-4 µm diam., by SEM perforated, greyish yellow (90 gy. Y) to brilliant yellow (83. brill. Y) by LM, branched, forming a lax net, with few free ends, ornamentation very irregular, some tubules with spines 0.5-3 µm long (Figs. 18-19, 21), others with granules, or a pitted reticulum (Fig. 20), the pits up to 3 µm diam. and then visible at high magnification by LM (Fig. 12), with a second faint reticulum below only visible by SEM (Fig. 20); the tubules with triangular expansions up to 15 µm wide at the junction of the branches, and sometimes with intercalated or terminal sub-globose, ellipsoid or fusiform expansions, 7.5-16 × 10-16 µm. Spores free, orange yellow (67. brill. OY) in mass, light green yellow (101. l. g Y) to brilliant yellow (83. brill. Y) by LM, sub-globose, 10-13.5 µm diam., densely warted, with flattened warts by SEM (Fig. 22). Plasmodium unknown.

Holotype: ARGENTINA. **Salta:** Molinos, Seclantás, RN-40 road, km 4467, 25º21'47"S 66º16'52"W, 2238 ± 6 m, dead leaf base of *Puya* sp., 29-III-2007, leg. *C. Lado, A. Estrada* and *D. Wrigley de Basanta*, dwb 2857 (MA-Fungi 78697).

Etymology: Named after Francisco de Diego Calonge, a renowned Spanish mycologist.

Habitat: Dead leaves of *Puya* spp.

Known distribution: northwest Argentina (states of Jujuy, Salta, Catamarca. La Rioja and San Juan). Possibly occurring in other areas of South America, following the distribution of species of the plant genus *Puya.*

Other specimens examined

ARGENTINA. **Catamarca:** Belén, RN-40 road to Hualfín, at 4 km from Belén, 27º36'55"S 67º01'06"W, 1305 ± 6 m, leaves of *Puya* sp., 27-XI-2006, Lado 18300 (MA-Fungi 78682), Lado 18301 (MA-Fungi 78683), Lado 18307 (MA-Fungi 78684). Belén, RN-40 road to Hualfín, at 7 km from Belén, Morro de los Cóndores Nature Reserve, 27º34'13"S 67º00'10"W, 1308 ±16 m, leaves of *Puya* sp., 27-XI-2006, Lado 18318 (MA-Fungi 78685), Lado 18321 (MA-Fungi 78686), Lado 18322 (MA-Fungi 78687), Lado 18330 (MA-Fungi 78688). Tinogasta, RN-60 road, km 1317, at 10 km from La Puntilla, 28°06'13"S 67°30'52"W, 1184 m ± 8m, dead leaf base of *Puya* sp., 18-III-2007, dwb 2838 (mc, pH 7.1). Tinogasta, Costa de Reyes, RP-3 road, 28°16'18"S 67°38'51"W, 1437 m ± 7m, dead leaf bases of *Puya* sp, 5-VII-2007, dwb 3009 (mc, pH 7.4); 29-XI-2007, leaves of *Puya* sp., Lado 18372 (MA-Fungi 78689). **Jujuy:** Tumbaya, Volcán, Huajra, 23°52'12"S 65°27'50"W, 2112 m ± 8m, dead leaf bases of *Puya* sp., 11-X-2007, dwb 2957 (mc, pH 6.9). **La Rioja:** Independencia, Talampaya National Park, RP-26 road, km 99, 30º07'42"S 67º44'19"W, 1378 ± 8 m, leaves of *Puya* sp., 30-XI-2006, Lado 18420 (MA-Fungi 78690). **Salta:** Molinos, Seclantás, RN-40 road, km 4467, 25º21'47"S 66º16'52"W, 2238 ± 6 m, leaves of *Puya* sp., 25-XI-2006, Lado 18236 (MA-Fungi 78678), Lado 18241 (MA-Fungi 78679), Lado 18242 (MA-Fungi 78680), Lado 18247 (MA-Fungi 78681); dead leaf base of *Puya* sp., 23-III-07, dwb 2833 (mc, pH 7), 29-III-2007, dwb 2857 (mc, pH 7) (holotype), 4-IV-2007, dwb 2865 (mc, pH 7.1). **San Juan:** Valle Fértil, Ischigualasto Provincial Park, RP-510 road to the Park, km 104, 30°10'44"S 67°48'56"W, 1374 m ± 12 m, dead leaf base of *Puya* sp., 23-III-2007, dwb 2850 (mc, pH 7.1), 14-IV-2007, dwb 2873 (mc, pH 6.9). San José de Jáchal, San Roque, RN-40 road, km 3619, 30º21'03"S 68º38'07"W, 1054 m, 8-III-2007, leaves of *Puya sp.,* Lado 18709 (MA-Fungi 78691), Lado 18710 (MA-Fungi 78692), Lado 18716 (MA-Fungi 78693). Ullum, RP-436 road, at 16.2 km northeast of the junction with RN-40 road, Termas de Talacasto, 31º01'41"S 68º45'44"W, 1333 m, 9-III-2007, leaves of *Puya sp.,* Lado 18723 (MA-Fungi 78694).

Ophiotheca wrightii Berk & M.A. Curtis, holotype K, Cuba, on wood, Coll. C. Wright 673 [A.L. 1713; ex herb. Berkeley].

Perichaena areolata Rammeloo, typus GENT, Rwanda, Mukavura-vulkaan (W. flank), 3500 m, on *Dendrosenecio* bladeren, 1/8/1974, Coll. Van der Veken, nr. 10581.

Discussion

Apart from the stalk, which in the genus *Perichaena* is not common, the most obvious character of this new *Perichaena* is the dark-edged polygonal peridial plates (Figs. 1-3, 5, 7) and the form of petaloid dehiscence of the peridium (Fig. 6). In addition, the capillitial tubules, with varied ornamentation from spiny to granulate and reticulate distinguish it from other species in the genus.

The spiny capillitium is similar to that of *Perichaena chrysosperma*, in which species some short-stalked sporocarps have been described, but the dehiscence of the peridium is irregular or longitudinal in this species (Rammeloo, 1984a), not by polygonal plates, and not leaving a petaloid calyculus-like base as in *P. calongei*. The capillitial spines in *P. calongei* are also shorter (0.5-3 µm long vs. 2.9-5.5 µm long in *P. chrysosperma),* less densely and not uniformly distributed, intercalated with many long stretches of totally spineless capillitium. In addition, the capillitium of *P. chrysosperma* has a pitted surface (Rammeloo, 1984a), and lacks the double reticulum of *P. calongei* by SEM (Figs. 20, 21).

The SEM illustrations (Rammeloo, 1984a) of the type of *Ophiotheca wrightii* Berk. & Curt., included in the species *Perichaena chrysosperma*, show capillitium with a reticulate surface. We examined the type specimen of *O. wrightii* preserved at K (*Wright 673*), which has definite sessile, flexuous plasmodiocarps, not sporocarps like *P. calongei*. The type material of *O. wrightii* has no peridial plates, nor outer markings on the peridium, unlike *P. calongei*, and spines 2.5-4 µm long, twice the diameter of the capillitial tubules (0.5-3 µm in *P. calongei*). By SEM, *O. wrightii* also has a very densely verrucose inner surface of the peridium (Rammeloo, 1984a), while *P. calongei* has an almost smooth inner surface, only faintly stippled by SEM (Fig. 17).

Other *Perichaena* species with short non-calcareous stalks are *P. areolata* Rammeloo, *P. pedata* and *P. reticulospora*. We examined the type specimen of *Perichaena areolata* (GENT 10581) which contains about 15 sporocarps either sessile or shortly stipitate. The closed sporocarps have a mottled peridial surface (Fig. 4) but no sign of peridial plates. The open sporocarps show irregular dehiscence. *Perichaena calongei* has a peridium divided into plates with a darker border (Figs. 1-3), and the open sporocarps show dehiscence by these plates (Figs 5-6). *Perichaena areolata* has a dense regular papillate ornamentation on the inner surface of the peridium by SEM (Rammeloo, 1984b) whereas *P. calongei* has an almost smooth inner surface to the peridium by SEM (Fig. 17). The ornamentation of the capillitial tubules is also different, composed of "spine-like excrecences up to 1.8 µm high" (Rammeloo, 1984a), which are regularly distributed in *P. areolata*, and varied ornamentation from spines, 0.5-3 µm long (Figs. 10, 13, 18, 19, 21), to granules (Fig. 11), with a pitted double reticulum (Figs. 12, 20), in *P. calongei*. The colour of the sporocarps is also different, a light brown to yellow in *P. aereolata* (Fig. 4) and dark brown to orange yellow in *P. calongei* (Figs. 1-3). *Perichaena pedata* is distinguished from *P. calongei* by the single vs. double peridium, by the ornamentation of the interior of the peridium, marked with dense verrucae by LM, short rounded low ridges in an incomplete network and with numerous verrucate elements by SEM (Rammeloo, 1984b), almost smooth in *P. calongei*. The capillitial tubules of *P. pedata* does not have a reticulate surface like that of *P. calongei* (Fig. 20), but has papilla-like excrescences (Rammeloo, 1984b) regularly distributed. *Perichaena reticulospora* has a banded-reticulate ornamentation on the spores (Keller and Reynolds, 1971), not warted like *P. calongei*.

Perichaena stipitata is clearly distinguishable by its white, calcareous stalk, and the single peridium (Estrada-Torres & al., 2009). Any specimens with limeless stalks, can be distinguished from *P. calongei* by the smooth unmarked surface of the sporotheca, the bright yellow colour of the sporotheca, the large perforations in the capillitial tubules with no reticulum and no spines, and the ocellate markings on the inside of the peridium.

More than a third of the known species of the genus *Perichaena* are stipitate, or occasionally have stalks, and half of these have been described in the last two years. In order to aid in the identification of these stipitate species a key has been proposed below, using characters from their original published descriptions as well as our own observations.

KEY TO THE *PERICHAENA* SPECIES

1. Spores polygonal ..... **P. polygonospora**
1. Spores globose or subglobose to ovate 2
2. Spores reticulate ..... **P. reticulospora**
2. Spores with different ornamentation but not reticulate 3
3. Spores ornamented with scattered, pyramid-like spines, around 1 µm high ..... **P. heterospinispora**
3. Spores densely ornamented with warts or spinules, less than 0.5 µm high 4
4. Spores 12-18 µm in diam. 5
4. Spores 7-12 µm in diam. 6
5. Stalk calcareous, white, spores 12-15 µm diam. ..... **P. stipitata**
5. Stalk not calcareous, brown or red brown, spores 14.5-18 µm diam. ..... **P. pulcherrima**
6. Peridium single 7
6. Peridium double 8
7. Sporotheca with an apical protuberance. Dehiscing leaving a disk-like basal part ..... **P. papulosa**
7. Sporotheca without an apical protuberance. Dehiscence irregular ..... **P. pedata**
8. Fructifications plasmodiocarpic to sessile sporocarps, occasionally mixed with short-stalked sporocarps. Capillitial tubules with spines, 2.9-5.5 µm long ..... **P. chrysosperma**
8. Fructifications not plasmodiocarpic, the sporocarps stipitate to sub-sessile. Capillitial tubules with spines up to 3 µm long 9
9. Peridium marked with dark lines, dehiscence along plates, capillitial tubules with spines, granules (Fig. 11) or a pitted reticulum ..... **P. calongei**
9. Peridium not marked with dark lines, dehiscence not along plates, capillitial tubules with regularly distributed spine-like excrecences ..... **P. areolata**

In moist chamber culture the new species appeared properly matured, after a mean incubation period of 25 days, in 8 out of the 33 cultures (24%) set up with the dead leaf bases of *Puya* species from Argentina. Three more collections were immature. The mean pH of the substrate producing this species in moist chamber culture, at 24 hours, was almost neutral (7.06). The bromeliad *Puya* grows on the ground in dense patches on the drier rocky slopes of these arid areas in Argenti-

Fig. 23. A species of the bromeliad genus *Puya* showing the dead leaves that serve as a substrate for *Perichaena calongei.*

na (Fig. 23). It has proved to be an excellent substrate for myxomycetes as cultures of the leaf bases have been over 94% positive for myxomycete fruiting bodies or plasmodia. No other substrate, of the more than 100 moist chamber cultures, prepared with native plant remains from the same areas produced this species. It therefore appears to have microhabitat requirements found so far only in this plant genus.

In the field the tiny sporocarps were found among the dead leaves at the base of the plant rosettes, where some moisture still remained. In moist chamber culture, as well as in the field, the sporocarps were scattered, often mixed on the same piece of substrate with other *Perichaena* species and myxomycetes of different genera such as *Cribraria*, *Arcyria*, and a newly described *Didymium* (Wrigley de Basanta & al., 2009).

The distinct morphological characters of *Perichaena calongei* were constant in both field and moist chamber collections. It was collected on several occasions over two years in five different states of Argentina and is thus described as a new species.

Acknowledgements

This research has been supported by the Ministry of Science and Innovation of Spain (grant CGL2005-00320/BOS and CGL2008-00720/BOS). We are very grateful to Fr. J. Bozonnet for his comments on the Latin diagnosis.

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Received: 3-III-2009
Accepted: 7-IV-2009

Resumen y Discusión General

A lo largo de los capítulos de esta memoria se han realizado distintas aproximaciones a la diversidad, ecología, y biogeografía de dos grupos de eumicetozoos: los protostélidos y los mixomicetes. Para ello se ha hecho hincapié en la influencia de diferentes factores ambientales sobre la distribución geográfica de estos organismos, tanto a nivel de morfoespecie como a nivel de cepa. En primer lugar se ha catalogado la diversidad de amebas protosteloides encontradas en la Península Ibérica. Posteriormente y tras optimizar los métodos de cultivo, se han estudiado los efectos del clima y de los microhábitats sobre la distribución de un conjunto de morfoespecies de protostélidos a escala Ibérica. A continuación se ha analizado la variabilidad genética del myxomycete *Badhamia melanospora*, estudiando los patrones de distribución geográfica de los grupos de ribotipos encontrados. También se ha descrito la variabilidad morfológica de las esporas que presenta cada grupo de ribotipos y se han comparado sus preferencias climáticas. Finalmente se ha descrito una especie nueva de myxomycete, perteneciente al género *Perichaena* (Trichiales).

Los resultados muestran que la diversidad de protostélidos en la Península Ibérica es comparable a la de otras zonas templadas muestreadas con anterioridad, y la intensidad del muestreo realizado ha permitido encontrar en esta zona casi la totalidad de las especies conocidas en el mundo. Tanto el tipo de microhábitat como el clima, tienen influencia sobre las comunidades de protostélidos, y cada una de las especies muestra distintas preferencias a este respecto.

Del estudio de *B. melanospora* a escala global se concluye que esta morfoespecie puede estar constituida por un complejo de especies crípticas. Las secuencias de ADN analizadas forman dos grupos de ribotipos geográficamente estructurados que también presentan diferencias morfológicas, aunque con rangos superpuestos, y ecológicas. El patrón de distribución de los ribotipos hace pensar que el aislamiento geográfico ha jugado un papel importante en la evolución de estos organismos en América. También sugiere que las poblaciones del Viejo Mundo pudieron ser introducidas por el hombre principalmente desde Norte América junto con las plantas suculentas que las albergan.

A continuación se discuten los resultados obtenidos.

Influencia de la falta de información previa en el estudio de los protostélidos

El estudio de la diversidad, distribución y ecología de las amebas protosteloides en la Península Ibérica ha estado condicionado por la falta de datos previos. Esta falta de conocimiento posiblemente se deba a que fueron descubiertos hace relativamente poco tiempo, ya que las primeras especies con este tipo de morfología fueron encontradas a comienzos de los años 60 (Olive & Stoianovitch, 1960; Olive, 1962), y fue durante esa década y la de los 70 durante las que se describieron la mayoría de las especies que se conocen hoy en dia.

Es destacable que con anterioridad al inicio de este estudio en 2006, no existía prácticamente ningún dato sobre estos organismos en el continente europeo, ya que tan sólo se habían publicado dos inventarios de especies realizados a nivel muy local en Alemania (Tesmer et al, 2005) y en Ucrania (Glutchenko et al, 2005). Al no haber sido tampoco estudiados hasta entonces en ninguna de las áreas con clima mediterráneo del mundo, en ese momento se desconocía por completo si los protostélidos podrían estar presentes en zonas con el clima y la vegetación típicas del suroeste de Europa. Tampoco se disponía de mucha información sobre su distribución a escala global, debido a que los esfuerzos por realizar inventarios de amebas protosteloides fuera de Norteamérica no comenzaron a intensificarse hasta pasados los años 90 (Stephenson et al, 1999; Moore & Spiegel, 2000a; Spiegel & Stephenson, 2000; Moore & Stephenson, 2003; Shadwick & Stephenson, 2004; Tesmer et al, 2005; Ndiritu et al, 2009a). Por tanto, las nuevas citas de protostélidos presentadas en esta memoria suponen ya de por sí una considerable mejora en el conocimiento corológico de estos organismos.

A pesar de aportaciones como la clasificación en grupos que realizó Spiegel (1990), y de recientes análisis filogenéticos basados en datos moleculares (Shadwick et al, 2009a; Lahr et al, 2011a), no está del todo claro todavía el grado de parentesco, si es que existe, entre las amebas protosteloides, ni su relación con otros grupos de eumicetozoos. Por tanto, en esta memoria se ha considerado a los protostélidos no como un taxón, sino como un conjunto de organismos que comparten una serie de características morfológicas y que constituyen un gremio ecológico, es decir, un grupo de organismos no necesariamente directamente relacionados entre sí, que comparten nichos ecológicos similares y explotan los mismos recursos.

El conocimiento de los requerimientos ambientales de los organismos eucariotas microscópicos es importante tanto para su estudio sistemático (Finlay, 2004), como para poder explicar sus patrones geográficos de distribución (Baas-Becking, 1934; Finlay, 2002). Desgraciadamente la ecología de las amebas protosteloides es otro aspecto de su estudio sobre el que existen pocos datos, y al se ha comenzado a dedicar atención sólo muy recientemente. En algunos casos las especies parecen tener preferencia por ciertos tipos de microhábitats o de climas (Spiegel et al, 2007), pero hasta ahora no se había podido estudiar con más detalle cuáles son las variables ambientales que más influyen sobre su distribución.

Los datos presentados en esta memoria constituyen una primera aportación al conocimiento sobre la distribución y ecología

de estos organismos en la Península Ibérica. Estudiar una zona relativamente extensa como esta ha permitido evaluar el efecto de distintas variables climáticas sobre la presencia y abundancia de las especies de amebas protosteloides, y compararlo con el de los microhábitats estudiados. Sin embargo, abordar este problema requirió realizar varios pasos previos.

El primer paso necesario fue comprobar que estos organismos se encontraban presentes en la Península Ibérica en zonas con clima eurosiberiano, y cuáles eran los sustratos – especies vegetales, estado de descomposición, etc – sobre los que se presentaban (Capítulo 1), y posteriormente realizar esas mismas comprobaciones en ambientes mediterráneos (Capítulo 2). El siguiente paso fue establecer una metodología apropiada para nuestros objetivos, que permitiera obtener datos informativos y comparables en todo el área de estudio optimizando el esfuerzo y ensayar distintas aproximaciones analíticas (Capítulo 2).

Por último, utilizando la metodología establecida se pudo completar el muestreo a una mayor escala y analizar los datos para obtener resultados finales (Capítulos 3 y 4).

Optimización del método de cultivo

A la hora de recoger las muestras, se consideró importante obtener una representación amplia de los protostélidos presentes en cada localidad (Figura 1). Por tanto se recolectaron muestras de tres microhábitats distintos: hojarasca del suelo, hojarasca aérea adherida a las plantas y corteza de plantas vivas. Estos microhábitats fueron escogidos por haber sido los más frecuentemente estudiados con anterioridad (Best & Spiegel, 1984; Moore & Spiegel, 2000a; Stephenson et al, 2004; Ndiritu et al, 2009a), por lo que podríamos comparar nuestros resultados con los de estudios anteriores. También se trató de estandarizar al máximo el tipo de muestras recogidas en cada localidad. El objetivo era recolectar siempre que fuera posible en cada sitio 4 muestras de hojarasca del suelo, 4 de hojarasca adherida a las plantas y 2 muestras de corteza. Además, en cada localidad se trató de recoger preferentemente muestras de gramíneas, fagáceas, genisteas, labiadas y cistáceas. Las cortezas se recogieron en los árboles y/o arbustos dominantes.

Como se comprobó en los primeros cultivos, al existir una serie de especies de protostélidos muy comunes (*Protostelium mycophagum*, *Schizoplasmodiopsis pseudoendospora*, *Tychosporium acutostipes*...) que aparecen en casi todas las localidades, era importante realizar un muestreo suficientemente intensivo como para detectar también las especies menos frecuentes, que sí mostraban mayores diferencias entre localidades. Al mismo tiempo, existía un conjunto de especies muy raras (*Schizoplasmodiopsis variabilis*, *Schizoplasmodium obovatum*, *Schizoplasmodium sechellarum*) y tan escasas que incluso con un esfuerzo muy alto tendrían muy pocas oportunidades de aparecer. Para solucionar este problema se realizó la optimización del esfuerzo de cultivo empleado por cada muestra que ha sido presentada en el Capítulo 2. Mediante esta optimización fue posible reducir el volumen de trabajo por muestra, permitiendo al mismo tiempo obtener los datos necesarios en localidad.

Tras realizar esta optimización, se observó que el tiempo que era necesario dedicar

a cada muestra y por tanto a cada localidad continuaba siendo elevado. Debido a que ya se había realizado gran parte del muestreo en el cuadrante nororiental y el centro de la península con una densidad elevada de puntos, y que estudiar el resto de la península con esa intensidad era inviable en el tiempo disponible, se optó por completar el muestreo en forma de transecto diagonal hacia el cuadrante suroccidental de la península, manteniendo una densidad de localidades similar.

La diversidad de amebas protosteloides en la Península Ibérica

Como resultado del muestreo realizado se encontraron 26 especies del total de 33 especies de protostélidos descritos (Capítulo 4). Por tanto la Península Ibérica constituye una de las zonas más ricas en especies de protostélidos que se conocen (Tabla 1). El número de especies encontradas en Somiedo (21) y en el centro de la península (18) son unos de los más altos encontrados a nivel local hasta la fecha. Y el total de especies encontradas en la península (26) supera al de todos los estudios publicados hasta ahora.

Al comparar los resultados presentados en esta memoria con los datos previos disponibles, las abundancias relativas de las especies encontradas no difieren mucho de las que aparecieron en otras zonas con climas templados (Tabla 1). Sin embargo, estas comparaciones deben realizarse con precaución porque en cada uno de dichos estudios se emplearon diferentes criterios para cuantificar la abundancia de las especies. En general una alta riqueza de especies como la encontrada aquí suele aparecer en zonas templadas, mientras que en estudios realizados en zonas de clima boreal o tropical suelen encontrarse un menor número de especies. Al igual que en la mayoría de los estudios anteriores *Protostelium mycophagum* y *Schizoplasmodiopsis pseudoendospora* se encuentran entre las especies más frecuentes también en la Península Ibérica. Otras especies, como *Cavostelium apophysatum*, *Protosporangium articulatum*, *Schizoplasmodiopsis amoeboidea* y *Tychosporium acutostipes* presentan una abundancia relativa mayor que en otras zonas de clima templado, al contrario que *Soliformovum irregularis*, que parece ser comparativamente menos frecuente en la Península Ibérica.

Las especies de protostélidos que no han sido encontradas durante nuestros muestreos son las siguientes:

• *Protosporangium conicum* W.E.Benn. Esta especie suele ser más frecuente que otros *Protosporangium* que sí se han encontrado en la Península Ibérica. Debido a que aparece exclusivamente en cortezas y es más frecuente en zonas áridas (Spiegel et al, 2007), es posible que no la hayamos encontrado por no haber muestreado con suficiente intensidad los ambientes en los que típicamente vive.

• *Protosteliopsis fimicola* (L.S.Olive) L.S.Olive & Stoian. Aunque también puede encontrarse en otros ambientes, esta especie suele aparecer mayoritariamente sobre excrementos de animales (Spiegel et al, 2007), sustratos que no han sido contemplados en nuestro muestreo.

• Por último, *Schizoplasmodiopsis variabilis* L.S. Olive, *Schizoplasmodium obovatum* L.S.Olive & Stoian. y *Schizoplasmodium sechellarum* L.S.Olive

Figura 1 – Ejemplo de localidad muestreada, situada en la provincia de Soria. En la mayoría de los modelos de nicho ambiental de protostélidos que han sido elaborados, esta zona de la península muestra una alta probabilidad de presencia de las especies.

& Stoian. son especies extremadamente raras y difíciles de encontrar (Spiegel et al, 2007).

Son problemáticos los pares formados por *Nematostelium gracilis* (L.S.Olive & Stoian.) L.S.Olive & Stoian./ *Ceratiomyxella tahitiensis* L.S.Olive & Stoian. y P*rotostelium mycophagum* L.S.Olive & Stoian./ *Planoprotostelium aurantium* L.S.Olive & Stoian., puesto que presentan una idéntica morfología en sus esporocarpos y podrían tratarse de una misma especie o bien formar parte de complejos de especies (Spiegel et al, 2007). Los caracteres que permiten distinguir a un organismo del otro aparecen exclusivamente en algunos estados tróficos de sus ciclos vitales, por lo que con la metodología que hemos empleado (identificación basada en la morfología de los esporocarpos) no es posible diferenciarlas. Para poder obtener identificaciones más fiables, hubiera sido necesario realizar aislamientos de las colonias de cuerpos fructíferos encontradas para observar la presencia o no de células flageladas en sus ciclos vitales y comprobar si existen diferencias mediante estudios moleculares. Realizar este tipo de trabajo habría requerido una cantidad de tiempo no razonable, y habría impedido alcanzar el resto de objetivos planteados en el tiempo disponible.

El desarrollo en el futuro de nuevas técnicas como la secuenciación de ADN de muestras ambientales (metagenómica) (Win Ko Ko et al, 2009; Kamono & Fukui, 2006; Kamono et al, 2009a, b) podrá permitir una mayor sensibilidad, fidelidad y precisión a la hora de detectar e identificar amebas protosteloides y otros eumicetozoos en las muestras, permitiendo así distinguir con mayor fiabilidad las especies con morfologías similares, y haciendo posible estudiar la "biodiversidad oculta" o el conjunto de organismos que no pueden ser detectados mediante cultivos. La puesta a punto y aplicación de este tipo de técnicas es sin duda alguna un próximo paso necesario para continuar el estudio de la composición y estructura de las comunidades de organismos presentes en estos ambientes.

Un acercamiento a la ecología de los protostélidos

Los organismos de pequeño tamaño, como los protostélidos, debido a sus elevados números poblacionales y a su eficaz dispersión, tienen típicamente distribuciones amplias y una baja tasa de endemismo (Finlay, 2002; Finlay & Fenchel, 2004). Los datos disponibles sobre las amebas protosteloides parecen confirmar este patrón, ya que la mayor parte de las especies han sido encontradas en localidades muy alejadas entre sí, y en distintos continentes (Tabla 1).

Como se ha explicado en la introducción general de esta memoria, según la hipótesis de "todo está en todas partes" el principal factor que seleccionaría qué organismos microscópicos estarían presentes en una determinada localidad sería la idoneidad ecológica de dicho ambiente para su supervivencia (Finlay, 2002). Sin embargo, la hipótesis alternativa de "endemismo moderado" defiende que, bien sea por la acción de barreras geográficas o factores históricos, o porque la dispersión no sea equiprobable en todas las direcciones, o porque no ocurra de manera instantánea y no ha transcurrido el tiempo necesario (Medlin, 2007; Foissner, 2009), existen al menos ciertos casos en los que la distribución de los organismos esta restringida a determinadas regiones (Foissner, 2006, 2009).

En cualquier caso, debido a la alta capacidad dispersiva de estos organismos, conocer sus preferencias ecológicas y localizar en qué zonas se encuentran los hábitats potencialmente adecuados para su supervivencia puede ser de gran utilidad para testar hipótesis biogeográficas. Por ello, uno de los objetivos planteados en esta memoria ha sido explorar la influencia de un conjunto de variables ambientales sobre las amebas protosteloides, y estudiar cómo dichas variables pueden influir sobre su distribución geográfica. El trabajo se ha centrado en el efecto causado por la variabilidad climática de la Península Ibérica sobre la abundancia de las especies presentes en tres microhábitats: hojarasca del suelo, hojarasca adherida a las plantas y corteza de plantas vivas.

Los resultados expuestos en el Capítulo 2, han mostrado que el tipo de microhábitat tiene una influencia muy importante para diferenciar los nichos de los protostélidos en la zona del centro peninsular estudiada. A esa escala también ciertas variables climáticas parecen tener efecto sobre la abundancia de las especies. Las que tuvieron mayor influencia fueron la temperatura mínima del mes más frío, la estacionalidad de las precipitaciones y el rango anual de temperatura. También se observó cierta correlación entre la preferencia por los distintos microhábitats y el clima. Las especies típicas de corteza de plantas vivas fueron más abundantes en las localidades con mayores rangos anuales de temperatura, y menor precipitación anual. Sin embargo, las especies con preferencia por la hojarasca adherida a las plantas aparecieron con mayor frecuencia en las zonas con mayor precipitación anual, menor temperatura del mes más cálido y menor temperatura del mes más frío.

En el Capítulo 3 se analizaron de nuevo los efectos del clima sobre las poblaciones de protostélidos en los distintos microhábitats, esta vez considerando un área de estudio mayor, en la que existe una variación climática más acusada entre las localidades. En este caso los efectos del clima sobre la abundancia de las especies de protostélidos encontradas cobran más fuerza. Las varia-

bles climáticas más importantes fueron la isotermalidad, los rangos anuales de temperatura, la estacionalidad de las temperaturas y la precipitación en los cuatro meses más fríos y más cálidos del año. Estos resultados y los del Capítulo 2 parecen indicar que en la Península Ibérica las variables que más contribuyen para diferencian los nichos de las especies son las que miden las variaciónes de temperatura y precipitación a lo largo del año.

También en este caso se encontró que existe una relación entre la preferencia por un determinado microhábitat y el tipo de clima. Las especies más abundantes en la hojarasca del suelo prefieren temperaturas altas y poca precipitación en invierno, mientras que las especies típicas de hojarasca adherida a la planta pueden tolerar menores temperaturas y mayor estacionalidad de la precipitación. Sin embargo, las relación entre las variables climáticas y microhábitats no es en este caso tan estrecha.

Los resultados presentados en esta memoria (Capítulos 2 y 3) y los datos anteriores disponibles muestran que la composición y la abundancia de especies en cada microhábitat varían junto con el clima. Se ha señalado con anterioridad que existe una cierta tendencia a que las mismas especies tiendan a vivir en la hojarasca del suelo en las zonas con mayor latitud, y en la hojarasca adherida a las plantas en las zonas con menor latitud. Una posible explicación puede ser que las condiciones microambientales se vean influidas por el clima externo. Sin embargo, con los métodos empleados no podemos llegar a conocer las condiciones diferenciales de cada microhábitat, por lo que no es posible comprobar la validez de esta hipótesis.

Los Modelos de Nicho Ambiental como herramienta para testar hipótesis

La escasez de citas y la falta de información sobre la ecología de las especies no es un problema que se encuentre exclusivamente durante el estudio de los protostélidos. De hecho, la mayoría de grupos de protistas cuentan con muy pocas citas, y los datos disponibles suelen estar fuertemente sesgados hacia determinadas zonas geográficas – generalmente Europa y Norte América – y determinados ambientes. La falta de este tipo de información hace muy difícil validar hipótesis relacionadas con la capacidad dispersiva o los patrones de distribución de estos organismos.

El uso de modelos de nicho ambiental puede ser de gran utilidad en el estudio de estos organismos, pues permite predecir la probabilidad de presencia de las especies en zonas sobre las que no se tienen suficientes datos, y ser usados como herramientas para ayudar a mejorar nuestro conocimiento sobre su ecología y su biogeografía. Conocer a priori las zonas donde existen hábitats adecuados para la supervivencia de las especies permitiría dar preferencia a los muestreos en esas zonas y comprobar si realmente los organismos se encuentran allí presentes.

Generamente, en la elaboración de los modelos no se tienen en cuenta algunos factores que pueder tener gran influencia sobre las especies, como variables microambientales, la interacción con otros organismos, la influencia humana, la existencia de barreras geográficas, etc. Como resultado los mapas obtenidos no muestran el nicho efectivo de las especies, sino que representan aproximaciones a su nicho fun-

damental. Sin embargo, conocer el nicho fundamental de algunas especies de protistas puede constituir una gran ventaja y servir como base para explorar el hipotético efecto causado por barreras geográficas y variables no incorporadas al modelo.

En el caso de los protostélidos ibéricos (Capítulo 3), los modelos obtenidos pueden ser útiles para predecir la probabilidad de encontrar las especies estudiadas en otras zonas mediterráneas no muestreadas hasta ahora, permitiendo con estos resultados mejorar el diseño de futuros muestreos.

Filogeografía de Badhamia melanospora

La aparición de diferencias en las preferencias ecológicas de los microorganismos puede tener gran importancia en su evolución. Se ha sugerido que la especialización ecológica podría ser una fuerza muy importante en el inicio de procesos de especiación en protistas (Finlay, 2004). En un escenario con dispersión global como el que plantea la hipótesis de "todo está en todas partes", o al menos con una dispersión limitada pero muy eficaz, los procesos de especiación simpátrica, fuertemente marcados por la adaptación ecológica, han de predominar por encima de la especiación alopátrica.

En el caso de *Badhamia melanospora*, los modelos de nicho ambiental fueron utilizados como una evidencia más de que la variabilidad genética de la especie muestra ciertos patrones geográficos, y que dicha variabilidad está relacionada con cambios en la morfología y la ecología de las especies. Los modelos predictivos obtenidos mostraron diferencias significativas entre los dos grupos de ribotipos, indicando que cada grupo genético podría haberse adaptado con mayor eficacia a las condiciones ecológicas locales de la zona que habita en América. La diferenciación genética de los grupos de ribotipos también parece haber conducido a un inicio en su diferenciación morfológica. Sin embargo, estos cambios morfológicos no son suficientes como para permitir discriminar a qué grupo pertenecen los organismos sin usar caracteres moleculares.

Tabla 3 (página siguiente) – Comparación entre estudios ecológicos de protostélidos en zonas templadas, tropicales y boreales, *: hábitat acuático, A: abundante (> 10%), C: común (> 5%), O: ocasional (> 1%), R: rara (< 1%), Ca: *Cavostelium apophysatum*, Cr: *Clastostelium recurvatum*, Ez: *Endostelium zonatum*, Eo: *Echinosteliopsis oligospora*, Eb: *Echinostelium bisporum*, Mp: *Microglomus paxillus*, Ng: *Nematostelium gracile*, No: *N. ovatum*, Pau: *Planoprotostelium aurantium*, Partic: *Protosporangium articulatum*, Pbi: *P. bisporum*, Pco: *P. conicum*, Pfr: *P. fragile*, Pf: *Protosteliopsis fimicola*, Pa: *Protostelium arachisporum*, Pm: *P. mycophagum* var. *mycophagum*, Pml: *P. mycophagum* "little", Pmr: *P. mycophagum* "repeater", Pmc: *P. mycophagum* var. *crassipes*, Pn: *P. nocturnum*, Po: *P. okumukumu*, Ppyr: *P. pyriforme*, Sa: *Schizoplasmodiopsis amoeboidea*, Sm: *S. micropunctata*, Sps: *S. pseudoendospora*, Sr: *S. reticulata*, Sv: *S. vulgaris*, Sc: *Schizoplasmodium cavostelioides*, Se: *Soliformovum expulsum*, Si: *S. irregulare*, Ta: *Tychosporium acutostipes*. (Modificado de Ndiritu et al, 2009a).

	ZONA DE ESTUDIO Y PUBLICACIÓN	RIQUEZA	ABUNDANCIA RELATIVA DE LAS ESPECIES																												
			Ca	Cr	Ez	Eo	Eb	Mp	Ng	No	Pau	Partic	Pbi	Pco	Pfi	Pf	Pa	Pm	Pml	Pmr	Pmc	Pn	Po	Ppyr	Sa	Sm	Sps	Sr	Sv	Sc	Se	Si	Ta
Zonas templadas	EEUU, Best & Spiegel (1984)	16	R			O	O		R				R		R	R	R	A				C		R		R	A			C		A	O
	EEUU, Moore & Spiegel (1995; 2000a, c)	16	R		R	O			C	C						R	R	A				R		R	R		A		O	C		A	O
	EEUU, Brown & Spiegel (2008)	22	O		O	C	O		O	O	R	O		O			C	A	A	O	O		O		O	A	R	A	R	C	O	A	O
	*EEUU, Lindley et al (2007)	10					O		A	A							O	A				A					A		A			A	A
	Alemania, Tesmer et al (2005)	15				R	R		C	C						R	R	A				R		R	O		A		C	R		A	R
	España, Aguilar et al (2007)	21	O		R	O	O	R	R	R					R		R	A				C	R	O	A	R	A		O	O	R	A	C
	India, Shadwick & Stephenson (2004)	12	R		O		R		R	O							R	A							C		A		R	C		O	
	*Alemania, Tesmer & Schmittler (2009)	12	O						C								R	O				O			A		A		O			O	A
	España, Aguilar et al (2011)	18	C		R	R	R		C	O		O					R	A				O	R	R	A		A		O	R		O	A
Zonas tropicales	Costa Rica, Stephenson & Moore (1998), Moore & Stephenson (2003)	16	A		R	O	A	R	C	C						R	C	C									A		R	R		O	R
	Puerto Rico, Stephenson et al (1999), Moore & Spiegel (2000b)	13	C			R			A	C							O	C						O	C		A		O	O		C	C
	Australia, Powers & Stephenson (2006)	12	C			A	O		A	O								A						O			A		C			C	R
	Tanzania, Ndiritu et al (2009)	14			O	O	O		O	R							R	A	A			R			O		O		R	C	O	C	R
	Malawi, Ndiritu et al (2009)	17	O		O	A	C	R	C	R					R		C	A	O			R			O		A		O	O	O	C	
	Kenya, Ndiritu et al (2009)	21	R	R	O	R	O	O	C	O		A				C	R	A	O	O	C	O		O	A		A		O	O	O	A	C
Zonas boreales	Alaska, Moore et al (2000)	8							R	O							R	C									A		C			R	O
	Isla de Macquarie, Spiegel & Stephenson (2000)	6																A				C			O	R	R					A	C
	Rusia, Kosheleva et al (2009)	14	R			R			C	O		A					O	A						O	C	R	A		C			O	O

En este sentido, Fiore-Donno et al (2011) han obtenido recientemente resultados similares al estudiar dos especies de *Lamproderma* estrechamente relacionadas. En su estudio, los genotipos encontrados también presentaban diferencias morfológicas con rangos de variabilidad superpuestos, no permitiendo indentificar claramente los organismos a priori y usando exclusivamente caracteres morfológicos. Estos autores han llegado a la conclusión de que gran parte de la variabilidad morfológica encontrada posiblemente puede estar producida por efectos microambientales durante la formación del esporocarpo.

El uso de caracteres moleculares en *B. melanospora* ha permitido estudiar esta morfoespecie con mayor sensibilidad y detectar diferencias y similitudes entre las muestras estudiadas que no serían perceptibles con el uso exclusivo de caracteres morfológicos. Nuestros resultados muestran que *B. melanospora* puede estar estar compuesta por un complejo de especies crípticas, pero con los datos disponibles es difícil establecer dónde están los límites entre las especies, pues no contamos con un concepto claro de especie ni para los protistas en general, ni para los mixomicetes en particular.

Los resultados obtenidos muestran que la dispersión de los propágulos de *B. melanospora* ha estado limitada entre las poblaciones norteamericanas y las procedentes de Argentina y Chile, lo que parece haber sido un factor importante en la diferenciación de linajes. Los grupos de ribotipos encontrados presentan un patrón geográfico congruente con la hipótesis de endemismo moderado, y son un ejemplo de cómo una morfoespecie aparentemente con una distribución amplia puede estar en realidad compuesta por una serie de líneas con una distribución restringida (Clark, 2004). En otros trabajos anteriores en los que se ha investigado la variabilidad intraespecífica en mixomicetes (Winsett & Stephenson, 2008; Fiore-Donno et al, 2011) no se encontró ninguna relación evidente entre distancia genética y distancia geográfica, aunque probablemente debido a que estos estudios fueron realizados con un número mucho menor de muestras o en un área geográfica más limitada.

Las secuencias de SSU procedentes de muestras no americanas se encuentran repartidas por diferentes clados en el árbol, pero generalmente relacionadas con poblaciones de América del norte. Estas muestras fueron encontradas en la mayoría de los casos sobre plantas suculentas introducidas originalmente desde el Nuevo Mundo. Nuestros datos sugieren que *B. melanospora* pudo ser traída junto con las plantas sobre las que fructifica al introducirlas en Europa, África, y las islas oceánicas estudiadas, por lo que este sería el primer caso documentado de introducción de mixomicetes por parte del hombre.

Descripción de una nueva especie de Perichaena

Se estima que tras 250 años de trabajo taxonómico tan sólo se han descrito un 14% de las especies de organismos terrestres y un 9% de los organismos marinos, y con las altas tasas de extinción actuales, es muy posible que muchos organismos desaparezcan antes de que lleguemos a conocer su existencia (Mora et al, 2011). Además, la catalogación de la biodiversidad está sesgada hacia las especies más conspícuas, con amplias distribuciones, mayor tamaño corporal y mayores abundancias (Gaston

& Blackburn, 2000; Mora et al, 2011). Por todo ello la descripción de nuevas especies, especialmente en los grupos de organismos menos estudiados, es un trabajo básico que debe ser realizado con urgencia.

Las especies del género *Perichaena* (Trichiales) se caracterizan principalmente por tener capilicios con fibras tubulares simples o ramificadas, rugosas, con verrugas, espinas o pequeños anillos, pero sin bandas espirales (Martin et al, 1983). Las fibras del capilicio tienen generalmente un contorno irregular y no son isodiamétricas a lo largo de su longitud. Normalmente estas fibras presentan perforaciones que sólo son visibles mediante microscopía electrónica de barrido. El género incluye 26 especies de las que sólo seis forman esporocarpos estipitados. Otras dos especies descritas como sésiles presentan estípites cortos o una base reducida que puede ser interpretada como un estípite muy corto.

En la nueva especie descrita, *Perichaena calongei*, la combinación de la morfología del esporocarpo, la estructura y la dehiscencia del peridio y la ornamentación del capilicio son diferentes de otras especies conocidas del género. Además de presentar estípite, lo que no es común en el género *Perichaena*, el carácter más llamativo en esta nueva especie es la presencia de peridios con placas poligonales con bordes oscuros y con dehiscencia petaloide. Además, las fibras del capilicio presentan una ornamentación variada, desde espinulosa a granulada. Se han encontrado esporocarpos de esta nueva especie tanto fructificados en el campo, como en cultivos en cámara húmeda de plantas recolectadas en las provincias de Catamarca, Jujuy, La Rioja, Salta y San Juan en Argentina.

Conclusiones

Las principales conclusiones que se pueden extraer de esta memoria son las siguientes:

1. Las amebas protosteloides se encuentran presentes en las zonas con clima mediterráneo del suroeste de Europa.

2. La optimización del esfuerzo de cultivo ha mostrado que empleando por cada muestra dos placas Petri con medio wMY con cuatro líneas de sustrato cada una, se obtiene al menos un 90% del total estimado de especies que podrían encontrarse en esta zona con esta misma metodología. Con una sola placa por muestra en las mismas condiciones se puede obtener el 80% del total estimado de especies.

3. Como resultado del muestreo realizado en España, Portugal y Francia se han encontrado 26 especies del total de 33 especies de protostélidos descritos en el mundo.

4. La distribución de las amebas protosteloides en la Península Ibérica no es totalmente al azar, sino que está determinada por las características del nicho de cada organismo, y la disponibilidad de hábitats apropiados para su supervivencia. Aunque las amebas protosteloides comparten similitudes morfológicas y tienen un modo de vida parecido, cada especie posee distintas preferencias ecológicas en cuanto al clima y los tipos de microhábitats que coloniza.

5. La composición y la estructura de las comunidades de protostélidos varían entre microhábitats tanto a escala local como a escala Ibérica.

6. Los cambios en la composición específica y la abundancia de las especies están relacionados con diferencias en el clima de las localidades de origen de las muestras. Estos efectos se hacen más visibles a escala Ibérica que usando exclusivamente los datos del centro de la Península.

7. En el análisis de correspondencias canónicas realizado con datos de toda la península las variables con mayor contribución son las que miden las variaciones de temperatura y precipitación a lo largo del año. Lo que diferencia los nichos de las especies es el tipo de cambio (temperatura, precipitación o ambos) y la magnitud de cambio que puede ser tolerado por cada una de ellas.

8. Las variables que miden cambios en la precipitación y la temperatura también fueron con mayor frecuencia las variables con más contribución en los modelos de nicho ambiental. La alternancia de estados ameboides con fructificaciones en los ciclos vitales puede suponer una ventaja para sobrevivir en ambientes cambiantes.

9. Los modelos de nicho ambiental realizados proporcionan estimaciones de la probabilidad de encontrar las especies de

amebas protosteloides estudiadas en zonas mediterráneas de la Península Ibérica usando la misma metodología que la empleada en este estudio. Según dichos modelos, las zonas con más probabilidad de presencia de protostélidos son las que presentan temperaturas medias anuales suaves, con un rango de temperaturas poco amplio y periodos de sequía poco pronunciados. Sin embargo, algunas especies muestran tolerancia por entornos más extremos.

10. El clima externo influye en la selección de microhábitats por parte de las especies. Las amebas protosteloides podrían tener diferentes rangos de tolerancia al clima en cada microhábitat, aumentando así su capacidad para colonizar rangos geográficos más amplios. Clarificar los patrones de interacción entre preferencias por los distintos microhábitats y el clima es esencial para entender la biogeografía de las amebas protosteloides.

11. En las zonas estudiadas la tendencia general es que los protostélidos más abundantes en la hojarasca del suelo prefieran zonas con temperaturas altas y poca precipitación en invierno. Las especies que aparecen con más frecuencia en la hojarasca adherida a las plantas pueden tolerar temperaturas más bajas y una mayor estacionalidad de las precipitaciones.

12. A la escala de este estudio, el efecto de los microhábitats es fuerte y comparable con los efectos del clima, pero se desconoce si su inflencia está determinada por las condiciones abióticas propias de cada microhábitat, o por el conjunto de otros organismos que pueden vivir allí e interaccionar con los protostélidos.

13. El estudio de los nichos ecológicos de las especies puede convertirse en una herramienta valiosa en el futuro de la biogeografía de protistas. Las hipótesis relacionadas con la ubicuidad o no de la dispersión de estos organismos no pueden ser facilmente falseadas sin un adecuado conocimiento previo de los requerimientos ecológicos de las especies.

14. *Badhamia melanospora* presenta una gran variabilidad en el fragmento de ADN que codifica para la subunidad pequeña del ribosoma (SSUr ADN) que se ha secuenciado, presentando un 16.3% de posiciones variables. Esta heterogeneidad define 32 ribotipos, que se dividen en dos grupos separados por 9 ribotipos ausentes.

15. *B. melanospora* tiene su origen más probable en Sudamérica.

16. La dispersión de los propágulos de *B. melanospora* ha estado limitada entre las poblaciones norteamericanas y las procedentes de Argentina y Chile, y que este hecho parece haber sido un factor importante en la diferenciación de linajes.

17. *B. melanospora* pudo haber llegado a Norteamérica mediate un único evento no reciente de colonización a larga distancia.

18. Los resultados muestran que *B. melanospora* pudo haber llegado a Europa, las islas oceánicas, África y Madagascar mediante múltiples eventos de colonización desde América. Las poblaciones de Norteamérica parecen haber sido la principal fuente para estas colonizaciones.

19. La dispersión de *B. melanospora* ha podido ser facilitada por el hombre mediante múltiples introducciones de plantas suculentas portadoras en el viejo mundo, siendo el primer caso documentado de introducción de mixomicetes por parte del hombre.

20. Las diferencias fenotípicas observadas son congruentes con las diferencias genéticas. Los grupos de ribotipos presentan esporas de distinto tamaño, aunque con rangos superpuestos, y diferencias en su ornamentación, aunque pueden encontrarse morfologías intermedias que podrían pertenecer a cualquiera de los dos grupos. Por tanto, los caracteres morfológicos estudiados no son útiles como caracteres diagnósticos, pero muestran que la diferenciación genética entre los grupos de ribotipos tiene algunas consecuencias sobre su morfología.

21. Los modelos de nicho ambiental realizados para los dos grupos de ribotipos presentan diferencias, y el grupo de ribotipos genéticamente menos diverso es el que posee un área predicha mas amplia. Por tanto, en el caso de *B. melanospora* la diversidad genética parece no estar relacionada con la amplitud de nicho ni implica una mayor capacidad colonizadora.

22. Esta morfoespecie parece estar compuesta por un complejo de especies, sobre el que han actuado la dispersión limitada, el aislamiento por distancia, la especificidad de sustratos y otros parámetros ecológicos, dando lugar a al menos dos especies crípticas aunque con ligeras diferencias morfológicas.

23. Los resultados obtenidos sugieren que considerando exclusivamente caracteres morfológicos, hay mucha información sobre las especies de mixomicetes que no puede ser percibida ni interpretada, y que los caracteres moleculares pueden ayudar a resolver numerosas preguntas sobre la biología de estos organismos en el futuro.

24. *Perichaena calongei* posee una combinación de caracteres morfológicos en sus fructificaciones, entre ellas la estructura y el tipo de dehiscencia del peridio, y la ornamentación del capilicio, que la distinguen del resto de las especies conocidas, por lo que se ha descrito como una especie nueva.

Glosario

afanoplasmodio – plasmodio delgado e inconspícuo, formado por un retículo de venas que producen frentes de avance en forma de abanico. El protoplasma realiza movimientos.

ameba – célula con forma irregular que se desplaza mediante pseudópodos.

ameba protosteloide – organismo que alterna en su ciclo de vida la presencia de estados ameboides o ameboflagelados con la formación de cuerpos fructíferos estipitados con una o muy pocas esporas

ameboflagelado – célula ameboide con uno o varios flagelos.

barrera geográfica – accidente geográfico que actúa como un obstáculo que impide o dificulta la dispersión de un organimo.

complejo de especies crípticas – conjunto de cepas que son morfológicamente indistinguibles o que presentan diferencias muy sutiles, pero que por su variabilidad genética pueden ser consideradas como especies independientes.

criptoespecie – ver *complejo de especies crípticas.*

cuerpo fructífero – estructura que contiene las esporas, formada a partir de una célula preesporal en el caso de protostélidos, a partir de un pseudoplasmodio en el caso de dictiostélidos, y a partir de un plasmodio en el caso de mixomicetes.

ecotipo – cepa genéticamente diferenciada que está adaptada a unas determinadas condiciones ambientales.

espora – célula reproductora que en los eumicetozoos posee paredes engrosadas y está asociada al cuerpo fructífero.

esporocarpo – cuerpo fructífero constituido por una estructura individualizada, como el de protostélidos, dictiostélidos y algunos mixomicetes.

faneroplasmodio – plasmodio grueso y macroscópico, compuesto por venas que forman un frente de avance en forma de abanico. El protoplasma realiza movimientos.

hongo mucilaginoso – organismo protista que alterna en su ciclo vital la formación de cuerpos fructíferos con estados ameboides o plasmodiales.

microhábitat – hábitat pequeño y localizado dentro de un ecosistema mayor, que posee características que mantienen un rango limitado de organismos que forman una comunidad diferenciada..

morfoespecie – especie definida teniendo en cuenta exclusivamente criterios morfológicos.

nicho efectivo – es la combinación de condiciones ambientales determinada por conjunto de variables tanto abióticas como bióticas que hacen posible la supervivencia

a largo plazo de una especie.

nicho fundamental – es la combinación de condiciones ambientales determinada por un conjunto de variables abióticas que hacen posible la supervivencia a lago plazo de una especie.

plasmodio – es una masa citoplasmática multinucleada no compartimentada por membranas celulares, es decir, que todos los núcleos comparten el mismo citoplasma. Se forma por la fusión de varias células o por la división repetidas veces del núcleo celular sin división del citoplasma.

protoplasmodio – plasmodio microscópico, similar a una ameba, sin venas ni reticulaciones.

protostélido – ver *ameba protosteloide.*

pseudoplasmodio – agregación de células que se reúnen pero no se fusionan, de forma que cada célula mantiene su independencia, y se desplazan conjuntamente.

pseudópodo – prolongación del citoplasma cubierta por la membrana celular usada por los organismos ameboides para alimentarse o desplazarse.

quiste – estructura de resitencia ante condiciones adversas que presenta paredes engrosadas.

subpseudópodo – estructura formada sobre un pseudópodo a modo de excrecencia.

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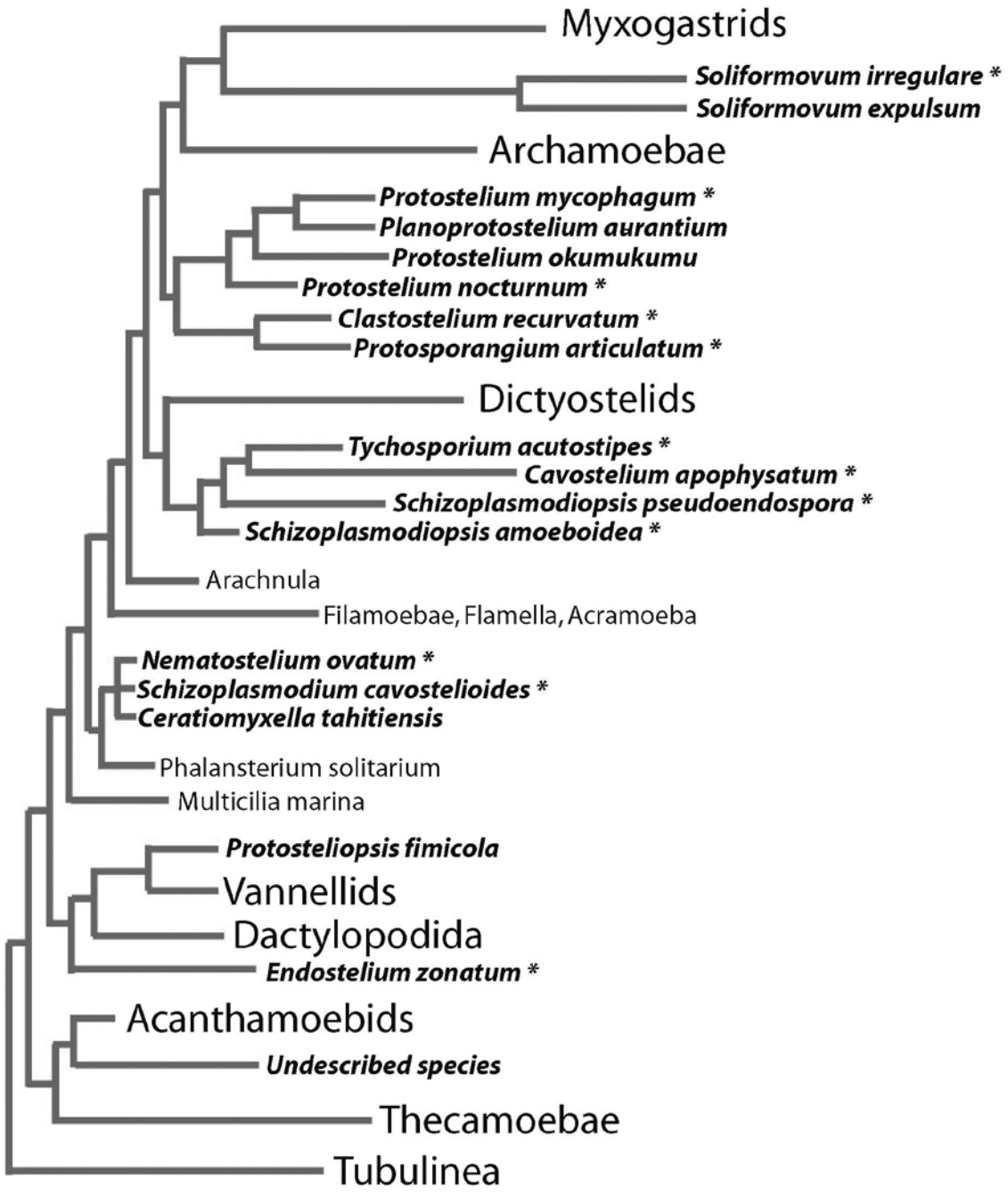
APÉNDICES

Apéndice 1:
Supplementary Material Cap. 3



Supplementary Figure S1 – Cartoon tree of Amoebozoa based on Shadwick et al (2009b). Protosteloid amoebae species are in italics, asterisks denote species included in this study.

Supplementary Table S2 – Species with more than 10 occurrences after equalizing the effort and coordinates of their localities in degrees. Ca: *Cavostelium apophysatum*, Ez: *Endostelium zonatum*, Ng: *Nematostelium gracile*, No: *Nematostelium ovatum*, Partic: *Protosporangium articulatum*, Pm: *Protostelium mycophagum*, Pn: *Protostelium nocturnum*, Ppyr: *Protostelium pyriforme*, Sa: *Schizoplasmodiopsis amoeboidea*, Sc: *Schizoplasmodium cavostelioides*, Si: *Soliformovum irregulare*, Sps: *Schizoplasmodiopsis pseudoendospora*, Sv: *Schizoplasmodiopsis vulgaris*, Ta: *Tychosporium acutostipes*.

SPECIES	LONGITUDE	LATITUDE
Ca	-6.14100	43.14636
Ca	-3.58778	40.81250
Ca	-3.43528	40.82556
Ca	-3.24417	40.69889
Ca	-2.73167	40.46000
Ca	-2.76306	40.29000
Ca	-2.73944	40.20167
Ca	-4.26389	40.41750
Ca	-4.36167	40.34028
Ca	-4.64444	40.21056
Ca	-4.84500	40.27056
Ca	-5.01194	40.11361
Ca	-2.41861	41.03944
Ca	-2.07556	41.01222
Ca	-1.17500	40.83278
Ca	-0.89667	40.71528
Ca	-0.14139	41.09333
Ca	0.02222	41.20750
Ca	-0.10472	41.36167
Ca	-0.14472	41.41139
Ca	-0.20750	41.62083
Ca	-0.39194	42.35361
Ca	-1.65389	42.29861
Ca	-2.58500	41.39417
Ca	-2.78222	39.86778
Ca	-1.89833	39.52806
Ca	-1.56222	39.59028
Ca	-1.31833	39.72639
Ca	-2.06194	40.57194
Ca	-1.56917	40.52250
Ca	-1.58444	40.43556
Ca	-3.77750	40.80500
Ca	-4.16056	40.59972
Ca	-6.88750	42.47278
Ca	-4.99500	42.99028
Ca	2.95556	42.44917
Ca	2.52556	42.15000
Ca	-5.39103	39.25938
Ca	-5.77551	38.61056
Ca	-6.34216	38.05285
Ca	-7.33106	37.41976
Ca	-5.27994	37.79406

Supplementary Table S2 – Cont.

Ez	-3.24417	40.69889
Ez	-4.26389	40.41750
Ez	-4.36167	40.34028
Ez	-4.64444	40.21056
Ez	-4.84500	40.27056
Ez	-5.01194	40.11361
Ez	-2.41861	41.03944
Ez	-0.39194	42.35361
Ez	-1.65389	42.29861
Ez	-2.78222	39.86778
Ez	-6.88750	42.47278
Ez	2.52556	42.15000
Ez	-8.27965	37.57528
Ng	-3.58778	40.81250
Ng	-3.43528	40.82556
Ng	-3.24417	40.69889
Ng	-2.73167	40.46000
Ng	-2.76306	40.29000
Ng	-2.73944	40.20167
Ng	-4.26389	40.41750
Ng	-4.36167	40.34028
Ng	-4.64444	40.21056
Ng	-4.84500	40.27056
Ng	-5.01194	40.11361
Ng	-2.41861	41.03944
Ng	0.02222	41.20750
Ng	-0.14472	41.41139
Ng	-0.20750	41.62083
Ng	-0.39194	42.35361
Ng	-0.32083	42.75694
Ng	-1.16000	42.61722
Ng	-1.35083	42.50778
Ng	-1.65389	42.29861
Ng	-2.74556	41.32222
Ng	-2.78222	39.86778
Ng	-1.89833	39.52806
Ng	-1.56222	39.59028
Ng	-1.31833	39.72639
Ng	-2.06194	40.57194
Ng	-1.56917	40.52250
Ng	-1.58444	40.43556
Ng	-3.74000	40.50167
Ng	-3.77750	40.80500
Ng	-4.16056	40.59972
Ng	-6.88750	42.47278
Ng	-3.04056	41.83611
Ng	2.52556	42.15000
Ng	-5.77551	38.61056
Ng	-6.34216	38.05285

Supplementary Table S2 – Cont.

Ng	-6.72140	37.92560
Ng	-5.27994	37.79406
No	-3.58778	40.81250
No	-3.24417	40.69889
No	-2.73167	40.46000
No	-4.26389	40.41750
No	-5.01194	40.11361
No	-0.10472	41.36167
No	-0.32083	42.75694
No	-1.65389	42.29861
No	-1.56222	39.59028
No	-1.31833	39.72639
No	-4.16056	40.59972
No	-4.99500	42.99028
No	-7.50581	37.92299
No	-8.28585	37.28218
Partic	-3.58778	40.81250
Partic	-3.24417	40.69889
Partic	-2.73167	40.46000
Partic	-2.73944	40.20167
Partic	-4.26389	40.41750
Partic	-4.36167	40.34028
Partic	-4.84500	40.27056
Partic	-2.07556	41.01222
Partic	0.02222	41.20750
Partic	-0.10472	41.36167
Partic	-0.20750	41.62083
Partic	-2.78222	39.86778
Partic	-1.89833	39.52806
Partic	-1.56222	39.59028
Partic	-1.31833	39.72639
Partic	-1.56917	40.52250
Partic	-3.77750	40.80500
Partic	2.95556	42.44917
Partic	-7.33106	37.41976
Partic	-5.27994	37.79406
Pm	-6.14100	43.14636
Pm	-6.20290	42.99541
Pm	-6.33294	43.14482
Pm	-3.58778	40.81250
Pm	-3.43528	40.82556
Pm	-3.24417	40.69889
Pm	-2.73167	40.46000
Pm	-2.76306	40.29000
Pm	-2.73944	40.20167
Pm	-4.26389	40.41750
Pm	-4.36167	40.34028
Pm	-4.64444	40.21056
Pm	-4.84500	40.27056

Supplementary Table S2 – Cont.

Ez	-3.24417	40.69889
Ez	-4.26389	40.41750
Ez	-4.36167	40.34028
Ez	-4.64444	40.21056
Ez	-4.84500	40.27056
Ez	-5.01194	40.11361
Ez	-2.41861	41.03944
Ez	-0.39194	42.35361
Ez	-1.65389	42.29861
Ez	-2.78222	39.86778
Ez	-6.88750	42.47278
Ez	2.52556	42.15000
Ez	-8.27965	37.57528
Ng	-3.58778	40.81250
Ng	-3.43528	40.82556
Ng	-3.24417	40.69889
Ng	-2.73167	40.46000
Ng	-2.76306	40.29000
Ng	-2.73944	40.20167
Ng	-4.26389	40.41750
Ng	-4.36167	40.34028
Ng	-4.64444	40.21056
Ng	-4.84500	40.27056
Ng	-5.01194	40.11361
Ng	-2.41861	41.03944
Ng	0.02222	41.20750
Ng	-0.14472	41.41139
Ng	-0.20750	41.62083
Ng	-0.39194	42.35361
Ng	-0.32083	42.75694
Ng	-1.16000	42.61722
Ng	-1.35083	42.50778
Ng	-1.65389	42.29861
Ng	-2.74556	41.32222
Ng	-2.78222	39.86778
Ng	-1.89833	39.52806
Ng	-1.56222	39.59028
Ng	-1.31833	39.72639
Ng	-2.06194	40.57194
Ng	-1.56917	40.52250
Ng	-1.58444	40.43556
Ng	-3.74000	40.50167
Ng	-3.77750	40.80500
Ng	-4.16056	40.59972
Ng	-6.88750	42.47278
Ng	-3.04056	41.83611
Ng	2.52556	42.15000
Ng	-5.77551	38.61056
Ng	-6.34216	38.05285

Supplementary Table S2 – Cont.

Ng	-6.72140	37.92560
Ng	-5.27994	37.79406
No	-3.58778	40.81250
No	-3.24417	40.69889
No	-2.73167	40.46000
No	-4.26389	40.41750
No	-5.01194	40.11361
No	-0.10472	41.36167
No	-0.32083	42.75694
No	-1.65389	42.29861
No	-1.56222	39.59028
No	-1.31833	39.72639
No	-4.16056	40.59972
No	-4.99500	42.99028
No	-7.50581	37.92299
No	-8.28585	37.28218
Partic	-3.58778	40.81250
Partic	-3.24417	40.69889
Partic	-2.73167	40.46000
Partic	-2.73944	40.20167
Partic	-4.26389	40.41750
Partic	-4.36167	40.34028
Partic	-4.84500	40.27056
Partic	-2.07556	41.01222
Partic	0.02222	41.20750
Partic	-0.10472	41.36167
Partic	-0.20750	41.62083
Partic	-2.78222	39.86778
Partic	-1.89833	39.52806
Partic	-1.56222	39.59028
Partic	-1.31833	39.72639
Partic	-1.56917	40.52250
Partic	-3.77750	40.80500
Partic	2.95556	42.44917
Partic	-7.33106	37.41976
Partic	-5.27994	37.79406
Pm	-6.14100	43.14636
Pm	-6.20290	42.99541
Pm	-6.33294	43.14482
Pm	-3.58778	40.81250
Pm	-3.43528	40.82556
Pm	-3.24417	40.69889
Pm	-2.73167	40.46000
Pm	-2.76306	40.29000
Pm	-2.73944	40.20167
Pm	-4.26389	40.41750
Pm	-4.36167	40.34028
Pm	-4.64444	40.21056
Pm	-4.84500	40.27056

Supplementary Table S2 – Cont.

Ez	-3.24417	40.69889
Ez	-4.26389	40.41750
Ez	-4.36167	40.34028
Ez	-4.64444	40.21056
Ez	-4.84500	40.27056
Ez	-5.01194	40.11361
Ez	-2.41861	41.03944
Ez	-0.39194	42.35361
Ez	-1.65389	42.29861
Ez	-2.78222	39.86778
Ez	-6.88750	42.47278
Ez	2.52556	42.15000
Ez	-8.27965	37.57528
Ng	-3.58778	40.81250
Ng	-3.43528	40.82556
Ng	-3.24417	40.69889
Ng	-2.73167	40.46000
Ng	-2.76306	40.29000
Ng	-2.73944	40.20167
Ng	-4.26389	40.41750
Ng	-4.36167	40.34028
Ng	-4.64444	40.21056
Ng	-4.84500	40.27056
Ng	-5.01194	40.11361
Ng	-2.41861	41.03944
Ng	0.02222	41.20750
Ng	-0.14472	41.41139
Ng	-0.20750	41.62083
Ng	-0.39194	42.35361
Ng	-0.32083	42.75694
Ng	-1.16000	42.61722
Ng	-1.35083	42.50778
Ng	-1.65389	42.29861
Ng	-2.74556	41.32222
Ng	-2.78222	39.86778
Ng	-1.89833	39.52806
Ng	-1.56222	39.59028
Ng	-1.31833	39.72639
Ng	-2.06194	40.57194
Ng	-1.56917	40.52250
Ng	-1.58444	40.43556
Ng	-3.74000	40.50167
Ng	-3.77750	40.80500
Ng	-4.16056	40.59972
Ng	-6.88750	42.47278
Ng	-3.04056	41.83611
Ng	2.52556	42.15000
Ng	-5.77551	38.61056
Ng	-6.34216	38.05285

Supplementary Table S2 – Cont.

Ng	-6.72140	37.92560
Ng	-5.27994	37.79406
No	-3.58778	40.81250
No	-3.24417	40.69889
No	-2.73167	40.46000
No	-4.26389	40.41750
No	-5.01194	40.11361
No	-0.10472	41.36167
No	-0.32083	42.75694
No	-1.65389	42.29861
No	-1.56222	39.59028
No	-1.31833	39.72639
No	-4.16056	40.59972
No	-4.99500	42.99028
No	-7.50581	37.92299
No	-8.28585	37.28218
Partic	-3.58778	40.81250
Partic	-3.24417	40.69889
Partic	-2.73167	40.46000
Partic	-2.73944	40.20167
Partic	-4.26389	40.41750
Partic	-4.36167	40.34028
Partic	-4.84500	40.27056
Partic	-2.07556	41.01222
Partic	0.02222	41.20750
Partic	-0.10472	41.36167
Partic	-0.20750	41.62083
Partic	-2.78222	39.86778
Partic	-1.89833	39.52806
Partic	-1.56222	39.59028
Partic	-1.31833	39.72639
Partic	-1.56917	40.52250
Partic	-3.77750	40.80500
Partic	2.95556	42.44917
Partic	-7.33106	37.41976
Partic	-5.27994	37.79406
Pm	-6.14100	43.14636
Pm	-6.20290	42.99541
Pm	-6.33294	43.14482
Pm	-3.58778	40.81250
Pm	-3.43528	40.82556
Pm	-3.24417	40.69889
Pm	-2.73167	40.46000
Pm	-2.76306	40.29000
Pm	-2.73944	40.20167
Pm	-4.26389	40.41750
Pm	-4.36167	40.34028
Pm	-4.64444	40.21056
Pm	-4.84500	40.27056

Supplementary Table S2 – Cont.

Ez	-3.24417	40.69889
Ez	-4.26389	40.41750
Ez	-4.36167	40.34028
Ez	-4.64444	40.21056
Ez	-4.84500	40.27056
Ez	-5.01194	40.11361
Ez	-2.41861	41.03944
Ez	-0.39194	42.35361
Ez	-1.65389	42.29861
Ez	-2.78222	39.86778
Ez	-6.88750	42.47278
Ez	2.52556	42.15000
Ez	-8.27965	37.57528
Ng	-3.58778	40.81250
Ng	-3.43528	40.82556
Ng	-3.24417	40.69889
Ng	-2.73167	40.46000
Ng	-2.76306	40.29000
Ng	-2.73944	40.20167
Ng	-4.26389	40.41750
Ng	-4.36167	40.34028
Ng	-4.64444	40.21056
Ng	-4.84500	40.27056
Ng	-5.01194	40.11361
Ng	-2.41861	41.03944
Ng	0.02222	41.20750
Ng	-0.14472	41.41139
Ng	-0.20750	41.62083
Ng	-0.39194	42.35361
Ng	-0.32083	42.75694
Ng	-1.16000	42.61722
Ng	-1.35083	42.50778
Ng	-1.65389	42.29861
Ng	-2.74556	41.32222
Ng	-2.78222	39.86778
Ng	-1.89833	39.52806
Ng	-1.56222	39.59028
Ng	-1.31833	39.72639
Ng	-2.06194	40.57194
Ng	-1.56917	40.52250
Ng	-1.58444	40.43556
Ng	-3.74000	40.50167
Ng	-3.77750	40.80500
Ng	-4.16056	40.59972
Ng	-6.88750	42.47278
Ng	-3.04056	41.83611
Ng	2.52556	42.15000
Ng	-5.77551	38.61056
Ng	-6.34216	38.05285

Supplementary Table S2 – Cont.

Ng	-6.72140	37.92560
Ng	-5.27994	37.79406
No	-3.58778	40.81250
No	-3.24417	40.69889
No	-2.73167	40.46000
No	-4.26389	40.41750
No	-5.01194	40.11361
No	-0.10472	41.36167
No	-0.32083	42.75694
No	-1.65389	42.29861
No	-1.56222	39.59028
No	-1.31833	39.72639
No	-4.16056	40.59972
No	-4.99500	42.99028
No	-7.50581	37.92299
No	-8.28585	37.28218
Partic	-3.58778	40.81250
Partic	-3.24417	40.69889
Partic	-2.73167	40.46000
Partic	-2.73944	40.20167
Partic	-4.26389	40.41750
Partic	-4.36167	40.34028
Partic	-4.84500	40.27056
Partic	-2.07556	41.01222
Partic	0.02222	41.20750
Partic	-0.10472	41.36167
Partic	-0.20750	41.62083
Partic	-2.78222	39.86778
Partic	-1.89833	39.52806
Partic	-1.56222	39.59028
Partic	-1.31833	39.72639
Partic	-1.56917	40.52250
Partic	-3.77750	40.80500
Partic	2.95556	42.44917
Partic	-7.33106	37.41976
Partic	-5.27994	37.79406
Pm	-6.14100	43.14636
Pm	-6.20290	42.99541
Pm	-6.33294	43.14482
Pm	-3.58778	40.81250
Pm	-3.43528	40.82556
Pm	-3.24417	40.69889
Pm	-2.73167	40.46000
Pm	-2.76306	40.29000
Pm	-2.73944	40.20167
Pm	-4.26389	40.41750
Pm	-4.36167	40.34028
Pm	-4.64444	40.21056
Pm	-4.84500	40.27056

Supplementary Table S2 – Cont.

Ez	-3.24417	40.69889
Ez	-4.26389	40.41750
Ez	-4.36167	40.34028
Ez	-4.64444	40.21056
Ez	-4.84500	40.27056
Ez	-5.01194	40.11361
Ez	-2.41861	41.03944
Ez	-0.39194	42.35361
Ez	-1.65389	42.29861
Ez	-2.78222	39.86778
Ez	-6.88750	42.47278
Ez	2.52556	42.15000
Ez	-8.27965	37.57528
Ng	-3.58778	40.81250
Ng	-3.43528	40.82556
Ng	-3.24417	40.69889
Ng	-2.73167	40.46000
Ng	-2.76306	40.29000
Ng	-2.73944	40.20167
Ng	-4.26389	40.41750
Ng	-4.36167	40.34028
Ng	-4.64444	40.21056
Ng	-4.84500	40.27056
Ng	-5.01194	40.11361
Ng	-2.41861	41.03944
Ng	0.02222	41.20750
Ng	-0.14472	41.41139
Ng	-0.20750	41.62083
Ng	-0.39194	42.35361
Ng	-0.32083	42.75694
Ng	-1.16000	42.61722
Ng	-1.35083	42.50778
Ng	-1.65389	42.29861

Supplementary Table S3 – Abundance of protosteloid amoeba in the selected localities after equalizing the effort. Coordinates are in degrees. LOC: locality, MH: microhabitat, A: aerial litter, G: ground litter, B: bark, Ca: *Cavostelium apophysatum*, Cr: *Clastostelium recurvatum*, Ea: *Endostelium amerosporum*, Eb: *Echinostelium bisporum*, Eo: *Echinosteliopsis oligospora*, Ez: *Endostelium zonatum*, Mp: *Microglomus paxillus*, Ng: *Nematostelium gracile*, No: *Nematostelium ovatum*, Pa: *Protostelium arachisporum*, Partic: *Protosporangium articulatum*, Pbisp: *Protosporangium bisporum*, Pm: *Protostelium mycophagum*, Pn: *Protostelium nocturnum*, Ppyr: *Protostelium pyriforme*, Sa: *Schizoplasmodiopsis amoeboidea*, Sc: *Schizoplasmodium cavostelioides*, Si: *Soliformovum irregulare*, Sm: *Schizoplasmodiopsis micropunctata*, Sps: *Schizoplasmodiopsis pseudoendospora*, Sr: *Schizoplasmodiopsis reticulata*, Sv: *Schizoplasmodiopsis vulgaris*, Ta: *Tychosporium acutostipes*.

LOC	COORDINATES		MH	ABUNDANCE OF THE SPECIES																					
	Y	X		Ca	Cr	Ea	Eb	Eo	Ez	Mp	Ng	No	Pa	Partic	Pbisp	Pm	Pn	Ppyr	Sa	Sc	Si	Sm	Sr	Sps	Sv	Ta
1	43.14636	-6.14100	S	1	0	0	0	1	0	0	0	0	0	0	0	2	0	0	2	1	0	0	0	1	2	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0	0	2	1	0
2	42.99541	-6.20290	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	1	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	1	0	0	0	0	0
			B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
3	43.14482	-6.33294	S	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	1	0	5	1	0	2	2	2
			A	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	1	0	0	0	1	0	1
			B	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
4	40.81250	-3.58778	S	6	0	0	0	0	0	0	1	0	0	0	0	4	0	0	2	0	0	0	0	8	1	3
			A	3	0	0	0	0	0	0	5	0	0	1	0	21	1	0	3	0	1	0	0	5	1	6
5	40.82556	-3.43528	S	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	2	0	0	0	0	5	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	1	0	0	0	0	3	0	3
6	40.69889	-3.24417	S	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	4	0	0
			A	0	0	0	0	0	0	0	0	0	0	1	0	9	0	0	1	0	0	0	0	3	0	0
7	40.46000	-2.73167	S	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	8	0	2
			A	2	0	0	0	0	0	0	0	1	0	0	0	12	1	0	3	0	1	0	0	3	1	6
8	40.29000	-2.76306	S	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	0	2
			A	0	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0
			S	4	0	0	0	0	0	0	0	0	0	0	0	5	0	0	5	0	0	0	0	0	0	5
9	40.20167	-2.73944	A	3	0	0	0	0	0	0	0	0	0	2	0	11	0	0	2	0	1	0	0	1	1	4
10	40.41750	-4.26389	S	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	2	0	2
			A	0	0	0	0	0	0	0	1	0	0	3	0	15	0	0	3	0	0	0	0	2	1	0
11	40.34028	-4.36167	S	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	4	0	1
			A	3	0	0	0	0	0	0	1	0	0	1	0	8	0	0	1	0	0	0	0	1	0	0

Supplementary Table S3 – Cont.

LOC	COORDINATES		MH	ABUNDANCE OF THE SPECIES																						
	Y	X		Ca	Cr	Ea	Eb	Eo	Ez	Mp	Ng	No	Pa	Partic	Pbisp	Pm	Pn	Ppyr	Sa	Sc	Si	Sm	Sr	Sps	Sv	Ta
1	43.14636	-6.14100	S	1	0	0	0	1	0	0	0	0	0	0	0	2	0	0	2	1	0	0	0	1	2	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0	0	2	1	0
2	42.99541	-6.20290	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	1	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	1	0	0	0	0	0
			B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
3	43.14482	-6.33294	S	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	1	0	5	1	0	2	2	2
			A	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	1	0	0	0	1	0	1
			B	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
4	40.81250	-3.58778	S	6	0	0	0	0	0	0	1	0	0	0	0	4	0	0	2	0	0	0	0	8	1	3
			A	3	0	0	0	0	0	0	5	0	0	1	0	21	1	0	3	0	1	0	0	5	1	6
5	40.82556	-3.43528	S	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	2	0	0	0	0	5	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	1	0	0	0	0	3	0	3
6	40.69889	-3.24417	S	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	4	0	0
			A	0	0	0	0	0	0	0	0	0	0	1	0	9	0	0	1	0	0	0	0	3	0	0
7	40.46000	-2.73167	S	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	8	0	2
			A	2	0	0	0	0	0	0	0	1	0	0	0	12	1	0	3	0	1	0	0	3	1	6
8	40.29000	-2.76306	S	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	0	2
			A	0	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0
			S	4	0	0	0	0	0	0	0	0	0	0	0	5	0	0	5	0	0	0	0	0	0	5
9	40.20167	-2.73944	A	3	0	0	0	0	0	0	0	0	0	2	0	11	0	0	2	0	1	0	0	1	1	4
10	40.41750	-4.26389	S	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	2	0	2
			A	0	0	0	0	0	0	0	1	0	0	3	0	15	0	0	3	0	0	0	0	2	1	0
11	40.34028	-4.36167	S	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	4	0	1
			A	3	0	0	0	0	0	0	1	0	0	1	0	8	0	0	1	0	0	0	0	1	0	0
12	40.21056	-4.64444	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	5	0	0
			A	3	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	1
			B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
13	40.27056	-4.84500	S	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	4	0	0
			A	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
			B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0

Supplementary Table S3 – Cont.

14	40.11361	-5.01194	S	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	3	0	3
			A	1	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1	0	0	0	0	0	0	6
15	41.03944	-2.41861	S	1	0	0	1	0	0	0	1	0	0	0	0	8	0	0	0	0	4	0	0	0	0	5
			A	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	1	0	0	0	0	0	0	6
			B	2	0	0	0	0	2	0	0	0	0	0	0	1	0	0	2	0	0	0	0	5	0	0
16	41.01222	-2.07556	S	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	4
			A	5	0	0	0	0	0	0	0	0	0	0	0	10	1	0	4	0	0	0	0	5	2	0
			B	3	0	0	0	0	0	0	0	0	0	2	0	9	0	0	4	9	6	0	0	2	0	3
17	40.83278	-1.17500	S	1	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	6	0	0	1	0	5
			A	0	0	0	0	0	0	0	0	0	0	0	2	11	1	0	1	0	15	0	0	0	0	2
			B	1	0	2	0	0	0	0	0	0	0	0	0	1	1	0	3	0	0	0	0	4	0	0
18	40.71528	-0.89667	S	1	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1	1	0	0	0	0	3
			A	0	0	0	0	0	0	0	0	0	0	0	0	11	0	1	0	0	0	0	0	0	0	0
19	40.80472	-0.67083	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			A	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
20	41.09333	-0.14139	S	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	10	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	23	1	0	2	0	2	0	0	7	0	9
			B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	3	0
21	41.20750	0.02222	S	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	10	1	0	0	15	0	5
			A	0	0	0	0	0	0	0	1	0	0	0	0	7	0	0	5	0	6	0	0	9	1	5
			B	4	0	0	1	0	0	0	0	0	0	4	0	0	0	0	12	3	0	0	0	0	0	0
22	41.41139	-0.14472	S	10	0	0	0	0	0	0	7	0	0	0	0	0	0	0	24	0	4	0	0	4	0	0
			A	7	0	0	0	0	0	0	4	0	0	0	0	18	0	0	13	5	0	0	0	0	0	5
23	41.62083	-0.20750	S	6	0	0	0	0	0	0	4	0	0	0	0	4	0	0	6	0	1	0	0	1	2	8
			A	1	0	0	0	0	0	0	0	0	0	0	0	9	2	0	2	4	0	0	0	0	0	2
			B	13	0	0	0	0	0	0	3	0	0	1	0	2	0	0	8	6	0	0	0	12	0	2
24	42.35361	-0.39194	S	1	0	0	0	0	0	0	2	0	0	0	0	6	0	0	2	0	0	0	0	0	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
			B	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	5	0	1
25	42.75694	-0.32083	S	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0
			A	0	0	0	0	0	0	0	1	0	0	0	0	7	1	0	0	0	0	0	0	0	0	0

Supplementary Table S3 – Cont.

LOC	COORDINATES		MH	ABUNDANCE OF THE SPECIES																					
	Y	X		Ca	Cr	Ea	Eb	Eo	Ez	Mp	Ng	No	Pa	Partic	Pbisp	Pm	Pn	Ppyr	Sa	Sc	Si	Sm	Sr	Sps	Sv	Ta
1	43.14636	-6.14100	S	1	0	0	0	1	0	0	0	0	0	0	0	2	0	0	2	1	0	0	0	1	2	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0	0	2	1	0
2	42.99541	-6.20290	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	1	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	1	0	0	0	0	0
			B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
3	43.14482	-6.33294	S	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	1	0	5	1	0	2	2	2
			A	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	1	0	0	0	1	0	1
			B	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
4	40.81250	-3.58778	S	6	0	0	0	0	0	0	1	0	0	0	0	4	0	0	2	0	0	0	0	8	1	3
			A	3	0	0	0	0	0	0	5	0	0	1	0	21	1	0	3	0	1	0	0	5	1	6
5	40.82556	-3.43528	S	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	2	0	0	0	0	5	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	1	0	0	0	0	3	0	3
6	40.69889	-3.24417	S	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	4	0	0
			A	0	0	0	0	0	0	0	0	0	0	1	0	9	0	0	1	0	0	0	0	3	0	0
7	40.46000	-2.73167	S	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	8	0	2
			A	2	0	0	0	0	0	0	0	1	0	0	0	12	1	0	3	0	1	0	0	3	1	6
8	40.29000	-2.76306	S	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	0	2
			A	0	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0
			S	4	0	0	0	0	0	0	0	0	0	0	0	5	0	0	5	0	0	0	0	0	0	5
9	40.20167	-2.73944	A	3	0	0	0	0	0	0	0	0	0	2	0	11	0	0	2	0	1	0	0	1	1	4
10	40.41750	-4.26389	S	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	2	0	2
			A	0	0	0	0	0	0	0	1	0	0	3	0	15	0	0	3	0	0	0	0	2	1	0
11	40.34028	-4.36167	S	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	4	0	1
			A	3	0	0	0	0	0	0	1	0	0	1	0	8	0	0	1	0	0	0	0	1	0	0
12	40.21056	-4.64444	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	5	0	0
			A	3	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	1
			B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
13	40.27056	-4.84500	S	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	4	0	0
			A	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
			B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0

Supplementary Table S3 – Cont.

14	40.11361	-5.01194	S	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	3	0	3
			A	1	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1	0	0	0	0	0	0	6
15	41.03944	-2.41861	S	1	0	0	1	0	0	0	1	0	0	0	0	8	0	0	0	0	4	0	0	0	0	5
			A	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	1	0	0	0	0	0	0	6
			B	2	0	0	0	0	2	0	0	0	0	0	0	1	0	0	2	0	0	0	0	5	0	0
16	41.01222	-2.07556	S	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	4
			A	5	0	0	0	0	0	0	0	0	0	0	0	10	1	0	4	0	0	0	0	5	2	0
			B	3	0	0	0	0	0	0	0	0	0	2	0	9	0	0	4	9	6	0	0	2	0	3
17	40.83278	-1.17500	S	1	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	6	0	0	1	0	5
			A	0	0	0	0	0	0	0	0	0	0	0	2	11	1	0	1	0	15	0	0	0	0	2
			B	1	0	2	0	0	0	0	0	0	0	0	0	1	1	0	3	0	0	0	0	4	0	0
18	40.71528	-0.89667	S	1	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1	1	0	0	0	0	3
			A	0	0	0	0	0	0	0	0	0	0	0	0	11	0	1	0	0	0	0	0	0	0	0
19	40.80472	-0.67083	S	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			A	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
20	41.09333	-0.14139	S	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	10	0	1
			A	0	0	0	0	0	0	0	0	0	0	0	0	23	1	0	2	0	2	0	0	7	0	9
			B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	3	0
21	41.20750	0.02222	S	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	10	1	0	0	15	0	5
			A	0	0	0	0	0	0	0	1	0	0	0	0	7	0	0	5	0	6	0	0	9	1	5
			B	4	0	0	1	0	0	0	0	0	0	4	0	0	0	0	12	3	0	0	0	0	0	0
22	41.41139	-0.14472	S	10	0	0	0	0	0	0	7	0	0	0	0	0	0	0	24	0	4	0	0	4	0	0
			A	7	0	0	0	0	0	0	4	0	0	0	0	18	0	0	13	5	0	0	0	0	0	5
23	41.62083	-0.20750	S	6	0	0	0	0	0	0	4	0	0	0	0	4	0	0	6	0	1	0	0	1	2	8
			A	1	0	0	0	0	0	0	0	0	0	0	0	9	2	0	2	4	0	0	0	0	0	2
			B	13	0	0	0	0	0	0	3	0	0	1	0	2	0	0	8	6	0	0	0	12	0	2

Supplementary Table S4 - Values of the 19 Bioclim variables in the selected localities. Coordinates are in degrees, temperatures are noted in ºC x 10, and precipitations in mm. LOC locality, AM annual mean temperature, DR mean diurnal range, IT isothermality, TS temperature seasonality, MTW maximum temperature of warmest month, mTC minimum temperature of coldest month, AR temperature annual range, TweQ mean temperature of wettest quarter, TDQ mean temperature of driest quarter, TWQ mean temperature of warmest quarter, TCQ mean temperature of coldest quarter, AP annual precipitation, Pwe precipitation of wettest month, PD precipitation of driest month, PS precipitation seasonality, PWeQ precipitation of wettest quarter, PDQ precipitation of driest quarter, PWQ precipitation of warmest quarter, PCQ precipitation of coldest quarter.

LOC	COORDINATES		VARIABLES																		
	Y	X	AM	DR	IT	TS	MTW	mTC	AR	TWeQ	TDQ	TWQ	TCQ	AP	PWe	PD	PS	PWeQ	PDQ	PWQ	PCQ
1	43.14636	-6.14100	113	101	42	4646	246	11	235	87	173	174	56	822	111	37	29	284	135	144	228
2	42.99541	-6.20290	62	100	40	5188	209	65495	250	7	131	131	1	1111	138	49	29	379	176	176	345
3	43.14482	-6.33294	117	98	42	4631	248	16	232	90	177	179	61	823	111	35	30	287	129	138	237
4	40.81250	-3.58778	127	102	35	6532	294	5	289	86	213	214	48	446	56	12	33	151	59	60	124
5	40.82556	-3.43528	120	104	36	6500	288	0	288	78	206	207	42	460	56	13	31	151	64	65	124
6	40.69889	-3.24417	125	104	35	6550	292	3	289	83	212	212	46	436	54	12	33	146	59	59	116
7	40.46000	-2.73167	127	112	37	6554	301	0	301	112	213	214	47	432	51	15	29	133	65	66	109
8	40.29000	-2.76306	127	115	37	6575	304	65534	306	112	214	215	47	445	52	14	30	138	64	65	115
9	40.20167	-2.73944	126	116	37	6624	305	65532	309	110	214	214	46	457	53	14	30	143	65	67	118
10	40.41750	-4.26389	129	106	35	6544	301	3	298	87	217	217	49	404	49	12	34	130	55	55	104
11	40.34028	-4.36167	138	108	35	6583	312	10	302	96	226	226	57	380	46	10	35	123	49	49	99
12	40.21056	-4.64444	139	112	36	6578	317	11	306	125	228	228	59	373	46	9	35	120	46	46	98
13	40.27056	-4.84500	136	113	37	6450	313	9	304	122	223	223	58	374	47	9	36	121	46	46	98
14	40.11361	-5.01194	145	114	37	6531	324	16	308	102	233	233	65	375	44	7	37	123	41	41	104
15	41.03944	-2.41861	101	113	38	6325	272	65512	296	121	185	186	25	518	66	25	24	164	94	103	124
16	41.01222	-2.07556	99	112	38	6286	265	65508	293	120	181	183	23	514	69	27	26	170	101	112	115
17	40.83278	-1.17500	92	98	36	6099	236	65505	267	112	17	172	17	508	74	26	33	184	89	134	89
18	40.71528	-0.89667	89	96	36	6081	234	65506	264	107	15	170	15	556	78	29	30	194	99	140	99
19	40.80472	-0.67083	98	94	36	6020	241	65516	261	116	26	178	26	525	73	27	31	181	92	129	92
20	41.09333	-0.14139	140	101	37	5901	287	16	271	143	76	217	68	434	58	18	32	139	79	88	80
21	41.20750	0.02222	152	104	37	5985	303	26	277	154	88	231	80	406	53	15	34	135	73	79	75
22	41.41139	-0.14472	139	104	37	6038	293	14	279	162	65	219	65	405	54	18	31	131	73	85	73
23	41.62083	-0.20750	128	104	37	5993	282	4	278	151	53	206	53	426	57	20	28	138	78	92	78
24	42.35361	-0.39194	75	95	38	5454	215	65501	250	92	147	147	8	837	95	49	16	246	181	191	193

Supplementary Table S4 – Cont.

25	42.75694	-0.32083	67	92	38	5313	200	65496	240	83	136	136	1	1031	108	63	13	289	223	236	253
26	42.69611	-0.52917	85	97	39	5349	222	65510	248	103	155	155	19	894	93	51	14	248	189	196	224
27	42.87556	-0.82472	67	94	38	5291	203	65497	242	12	137	137	3	1098	113	64	14	310	226	226	291
28	42.61722	-1.16000	115	103	39	5577	261	0	261	82	188	188	45	829	95	41	20	251	154	154	236
29	42.50778	-1.35083	119	105	39	5758	269	0	269	55	193	193	46	782	92	38	21	239	140	140	226
30	42.29861	-1.65389	128	106	38	5953	282	8	274	92	205	205	54	618	69	30	22	184	110	110	170
31	41.75139	-1.95472	93	111	38	6038	257	65506	287	112	172	173	19	608	76	34	22	193	120	134	148
32	41.39417	-2.58500	106	113	38	6242	278	65517	297	125	189	189	31	510	62	25	23	157	93	100	128
33	41.32222	-2.74556	104	112	37	6271	275	65516	295	123	187	188	28	520	63	24	23	159	92	98	130
34	39.86778	-2.78222	126	120	38	6646	310	65531	315	109	214	215	46	471	55	13	31	153	63	65	124
35	39.52806	-1.89833	128	120	39	6384	303	65534	305	111	213	214	52	450	54	15	29	148	71	77	101
36	39.59028	-1.56222	130	114	39	6285	296	4	292	148	214	215	55	436	53	16	29	141	76	80	91
37	39.72639	-1.31833	114	109	38	6268	276	65527	285	130	198	198	40	498	62	22	27	163	96	100	99
38	40.57194	-2.06194	95	114	37	6384	267	65502	301	114	180	181	18	553	73	27	25	181	103	115	124
39	40.52250	-1.56917	83	106	37	6288	245	65495	286	101	8	168	8	590	80	34	26	201	119	139	119
40	40.43556	-1.58444	88	108	37	6315	250	65500	286	107	13	173	13	566	77	31	27	194	113	131	113
41	40.50167	-3.74000	135	102	34	6610	305	12	293	92	223	223	53	449	57	11	37	157	52	52	134
42	40.80500	-3.77750	107	103	36	6411	275	65525	286	66	193	194	31	508	58	16	30	159	72	78	135
43	40.59972	-4.16056	93	106	36	6229	264	65512	288	109	178	178	21	527	68	20	31	168	80	86	128
44	42.47278	-6.88750	127	102	39	5438	272	14	258	64	198	198	59	734	97	19	40	277	80	80	261
45	42.69500	-6.92889	114	99	39	5194	255	6	249	54	182	182	50	855	112	25	38	318	102	102	297
46	42.85889	-6.88556	102	97	39	5076	242	65535	243	46	169	169	41	948	122	31	35	346	122	122	320
47	42.99028	-4.99500	89	101	40	5046	233	65519	250	64	155	156	28	825	102	43	24	275	154	155	220
48	42.90278	-4.80083	81	101	39	5145	228	65510	254	56	150	150	19	839	101	44	23	274	155	155	225
49	36.82778	-2.03917	179	82	37	4754	295	76	219	157	239	244	122	241	34	2	50	95	13	20	82
50	41.92667	-2.66000	93	112	38	5986	262	65510	288	110	173	173	21	637	73	34	19	189	120	120	167
51	41.83611	-3.04056	101	109	38	6027	268	65520	284	119	182	182	28	555	64	27	21	166	100	100	144
52	42.44917	2.95556	126	79	33	5434	255	19	236	97	197	198	60	788	97	39	21	235	164	171	178
53	42.15000	2.52556	125	75	32	5523	253	21	232	141	59	198	59	893	95	44	21	264	177	227	177
54	39.82415	-5.47966	158	115	37	6557	339	31	308	116	246	246	78	394	47	5	44	135	33	33	122
55	39.68413	-5.45146	141	115	36	6622	325	14	311	69	231	231	61	470	55	7	42	159	41	41	146
56	39.53715	-5.37449	141	117	37	6670	328	13	315	68	231	231	61	485	58	6	44	165	40	40	152

Supplementary Table S4 – Cont.

LOC	COORDINATES		VARIABLES																		
	Y	X	**AM**	**DR**	**IT**	**TS**	**MTW**	**mTC**	**AR**	**TWeQ**	**TDQ**	**TWQ**	**TCQ**	**AP**	**PWe**	**PD**	**PS**	**PWeQ**	**PDQ**	**PWQ**	**PCQ**
1	43.14636	-6.14100	113	101	42	4646	246	11	235	87	173	174	56	822	111	37	29	284	135	144	228
2	42.99541	-6.20290	62	100	40	5188	209	65495	250	7	131	131	1	1111	138	49	29	379	176	176	345
3	43.14482	-6.33294	117	98	42	4631	248	16	232	90	177	179	61	823	111	35	30	287	129	138	237
4	40.81250	-3.58778	127	102	35	6532	294	5	289	86	213	214	48	446	56	12	33	151	59	60	124
5	40.82556	-3.43528	120	104	36	6500	288	0	288	78	206	207	42	460	56	13	31	151	64	65	124
6	40.69889	-3.24417	125	104	35	6550	292	3	289	83	212	212	46	436	54	12	33	146	59	59	116
7	40.46000	-2.73167	127	112	37	6554	301	0	301	112	213	214	47	432	51	15	29	133	65	66	109
8	40.29000	-2.76306	127	115	37	6575	304	65534	306	112	214	215	47	445	52	14	30	138	64	65	115
9	40.20167	-2.73944	126	116	37	6624	305	65532	309	110	214	214	46	457	53	14	30	143	65	67	118
10	40.41750	-4.26389	129	106	35	6544	301	3	298	87	217	217	49	404	49	12	34	130	55	55	104
11	40.34028	-4.36167	138	108	35	6583	312	10	302	96	226	226	57	380	46	10	35	123	49	49	99
12	40.21056	-4.64444	139	112	36	6578	317	11	306	125	228	228	59	373	46	9	35	120	46	46	98
13	40.27056	-4.84500	136	113	37	6450	313	9	304	122	223	223	58	374	47	9	36	121	46	46	98
14	40.11361	-5.01194	145	114	37	6531	324	16	308	102	233	233	65	375	44	7	37	123	41	41	104

Supplementary Table 4 (next page) - Correlation between all pairs of the 19 Bioclim variables in the selected localities. Values correspond to the squared correlation coefficients (r2). Correlation coefficients higher than 0.9 are highlighted. AM: annual mean temperature, DR: mean diurnal range, IT: isothermality, TS: temperature seasonality, MTW maximum temperature of warmest month, mTC minimum temperature of coldest month, AR temperature annual range, TweQ mean temperature of wettest quarter, TDQ: mean temperature of driest quarter, TWQ: mean temperature of warmest quarter, TCQ: mean temperature of coldest quarter, AP: annual precipitation, Pwe: precipitation of wettest month, PD: precipitation of driest month, PS: precipitation seasonality, PWeQ: precipitation of wettest quarter, PDQ: precipitation of driest quarter, PWQ: precipitation of warmest quarter, PCQ: precipitation of coldest quarter, ***: $p < 0.0001$, **: $p < 0.001$, *: $p < 0.05$, #: $p < 0.1$, X: $p > 0.1$.

	DR	IT	TS	MTW	mTC	AR	TWeQ	TDQ	TWQ	TCQ	AP	PWe	PD	PS	PWeQ	PDQ	PWQ	PCQ
AM	0.02487 #	0.007592 X	-0.01307 X	0.7225 ***	0.5927 ***	0.003821 X	0.1378 ***	0.05672 *	**0.9142** ***	0.05729 *	0.2943 ***	0.1229 **	0.7538 ***	0.7076 ***	0.05144 *	0.7234 ***	0.7181 ***	-0.00953 X
DR		0.007342 X	0.5222 ***	0.3941 ***	-0.0125 X	0.8153 ***	-0.01336 X	0.08658 **	0.1468 ***	0.08861 **	0.1723 ***	0.1748 ***	0.1919 ***	0.00512 X	0.1375 ***	0.1897 ***	0.2315 ***	0.05055 *
IT			0.2904 ***	-0.01331 X	-0.01176 X	0.06916 *	-0.00814 X	0.08886 **	-0.0131 X	0.0891 **	0.02078 X	0.1204 **	-0.0059 X	0.08356 **	0.1504 ***	-0.0021 X	0.00865 X	0.2057 ***
TS				0.2359 ***	-0.00629 X	0.8902 ***	-0.00694 X	-0.00505 X	0.0833 **	-0.0046 X	0.2599 ***	0.4136 ***	0.079 **	0.00730 X	0.4091 ***	0.0709 *	0.07137 *	0.3425 ***
MTW					0.3335 ***	0.3807 ***	0.07045 *	0.0712 *	**0.9128** ***	0.07274 *	0.4512 ***	0.318 ***	0.8124 ***	0.4578 ***	0.203 ***	0.776 ***	0.8057 ***	0.04822 *
mTC						-0.01187 X	0.005132 X	0.005116 X	0.4913 ***	0.00523 X	0.0541 *	0.00597 X	0.2979 ***	0.3243 ***	-0.0098 X	0.2636 ***	0.278 ***	-0.00647 X
AR							-0.01075 X	0.0234 #	0.1482 ***	0.02451 #	0.26 ***	0.35 ***	0.1595 ***	-0.01351 X	0.3182 ***	0.1504 ***	0.1702 ***	0.2086 ***
TWeQ								0.007511 X	0.142 ***	0.00680 X	0.4097 ***	0.3334 ***	0.1214 **	0.00479 X	0.3649 ***	0.1286 ***	0.0784 **	0.416 ***
TDQ									0.0606 *	0.9999 ***	0.1006 **	0.04665 *	0.1291 ***	0.0164 X	0.0319 #	0.1217 **	0.1096 **	0.01333 X
TWQ										0.06155 *	0.4551 ***	0.2832 ***	0.8461 ***	0.5822 ***	0.1747 ***	0.8086 ***	0.8043 ***	0.0418 *
TCQ											0.1008 **	0.0471 *	0.1303 ***	0.01665 X	0.03209 #	0.1227 **	0.1112 **	0.0129 X
AP												0.8715 ***	0.5867 ***	0.09862 **	0.8128 ***	0.602 ***	0.5584 ***	0.6499 ***
PWe													0.3219 ***	-0.0099 X	**0.9687** ***	0.3217 ***	0.3085 ***	0.7901 ***
PD														0.6543 ***	0.2019 ***	**0.9899** ***	**0.9716** ***	0.06304 *
PS															-0.0087 X	0.6629 ***	0.6405 ***	0.03814 *
PWeQ																0.2053 ***	0.1891 ***	0.8926 ***
PDQ																	**0.9722** ***	0.06765 *
PWQ																		0.0409 *

Apéndice 2:
Supplementary Material Cap. 5

Supplementary Table S1 – Sample providers, locality data, and ribotype group. CL: C. Lado, LHC: L. H. Cavalcanti, SS: S. L Stephenson, MM: M. Meyer, RM: R. McHugh.

LOCALITY NO	SAMPLE	PROVIDER	Y	X
1	19481ARG	CL	-34.592	-69.029
1	19483ARG	CL	-34.592	-69.029
1	19486ARG	CL	-34.592	-69.029
2	15695CHL	CL	-34.081	-70.612
3	18695ARG	CL	-32.899	-66.741
4	17989CHL	CL	-32.870	-70.849
5	17940CHL	CL	-31.488	-71.098
6	17523CHL	CL	-31.254	-70.474
7	17985CHL	CL	-31.051	-71.600
8	17932CHL	CL	-30.499	-71.114
9	17522CHL	CL	-30.254	-70.474
10	17964CHL	CL	-30.010	-70.688
11	17980CHL	CL	-29.926	-70.535
12	15674CHL	CL	-29.361	-71.061
13	15679CHL	CL	-29.307	-71.280
13	15680CHL	CL	-29.307	-71.280
14	15668CHL	CL	-28.671	-70.771
15	18392ARG	CL	-28.609	-67.640
15	18393ARG	CL	-28.609	-67.640
16	15652CHL	CL	-28.251	-71.158
17	15638CHL	CL	-28.147	-71.064
18	18336ARG	CL	-27.753	-67.207
19	18294ARG	CL	-27.615	-67.018
20	15619CHL	CL	-26.156	-70.650
21	18269ARG	CL	-26.144	-65.958
22	18260ARG	CL	-25.491	-66.236
23	19986MDG	CL	-25.030	45.827
24	19879MDG	CL	-25.025	46.429
25	19914MDG	CL	-25.024	46.644
25	19916MDG	CL	-25.024	46.644
26	15613CHL	CL	-25.005	-70.406
27	19940MDG	CL	-24.995	46.523
28	19958MDG	CL	-24.976	46.234
29	19955MDG	CL	-24.971	46.233
30	19113CHL	CL	-24.962	-70.476
30	19117CHL	CL	-24.962	-70.476
31	15615CHL	CL	-24.896	-70.525
32	19989MDG	CL	-24.518	45.625
33	19859MDG	CL	-24.112	45.615

Supplementary Table S1 – Cont.

33	19860MDG	CL	-24.112	45.615
34	18133ARG	CL	-23.699	-65.548
34	18135ARG	CL	-23.699	-65.548
35	18090ARG	CL	-23.680	-65.447
35	18091ARG	CL	-23.680	-65.447
35	18092ARG	CL	-23.680	-65.447
35	18093ARG	CL	-23.680	-65.447
35	18095ARG	CL	-23.680	-65.447
35	18096ARG	CL	-23.680	-65.447
35	18097ARG	CL	-23.680	-65.447
35	18119ARG	CL	-23.359	-65.345
35	18120ARG	CL	-23.359	-65.345
35	18121ARG	CL	-23.359	-65.345
35	18129ARG	CL	-23.359	-65.345
36	19990MDG	CL	-23.182	46.053
36	19995MDG	CL	-23.182	46.053
37	19834MDG	CL	-22.397	46.119
38	15555CHL	CL	-18.343	-69.515
38	15560CHL	CL	-18.343	-69.515
39	15541CHL	CL	-18.327	-69.582
40	60295BRA	LHC	-8.000	-39.600
41	22029GBR	SS	-7.937	-14.372
42	60295BRA	LHC	-6.916	-38.770
43	15003MEX	CL	17.637	-96.917
43	15037MEX	CL	17.637	-96.917
44	14995MEX	CL	18.016	-97.056
45	10910MEX	CL	18.073	-97.352
46	14933MEX	CL	18.224	-97.455
46	14942MEX	CL	18.224	-97.455
46	14951MEX	CL	18.224	-97.455
46	14969MEX	CL	18.224	-97.455
47	10927MEX	CL	18.278	-97.328
48	14789MEX	CL	18.733	-97.530
49	14763MEX	CL	18.733	-97.544
50	14734MEX	CL	18.862	-97.605
50	14741MEX	CL	18.862	-97.605
50	14750MEX	CL	18.862	-97.605
51	12704MEX	CL	19.294	-97.506
52	10760MEX	CL	19.474	-97.924
53	12731MEX	CL	19.482	-97.367
53	12732MEX	CL	19.482	-97.367

Supplementary Table S1 – Cont.

53	12741MEX	CL	19.482	-97.367
53	12744MEX	CL	19.482	-97.367
53	12768MEX	CL	19.482	-97.367
54	11339MEX	CL	19.730	-98.712
55	13009MEX	CL	19.903	-98.704
56	35326MEX	MM	20.022	-98.638
57	12984MEX	CL	20.267	-99.180
58	11149MEX	CL	20.309	-99.861
59	11094MEX	CL	20.364	-99.043
59	11099MEX	CL	20.364	-99.043
60	12922MEX	CL	20.554	-99.163
61	11251MEX	CL	20.605	-99.105
61	11257MEX	CL	20.605	-99.105
62	15182MEX	CL	20.824	-100.057
63	18822MEX	CL	25.623	-102.884
64	18857MEX	CL	25.862	-103.773
64	18858MEX	CL	25.862	-103.773
65	18940MEX	CL	26.567	-103.966
66	13114ESP	CL	28.371	-16.384
67	879USA	SS	29.197	-103.029
68	807USA	SS	29.272	-103.159
69	35708MOR	MM	30.038	-9.639
70	35775MOR	MM	30.337	-9.516
71	35733MOR	MM	30.362	-9.508
72	35739MOR	MM	30.373	-9.558
73	35723MOR	MM	30.429	-9.617
74	35724MOR	MM	30.440	-9.641
75	35700MOR	MM	30.502	-9.596
76	35729MOR	MM	30.538	-9.696
77	35728MOR	MM	30.580	-9.738
78	35706MOR	MM	30.597	-9.517
79	35767MOR	MM	30.605	-10.023
80	35766MOR	MM	30.652	-9.876
81	35713MOR	MM	30.726	-7.944
82	35772MOR	MM	30.843	-9.498
83	21735MOR	MM	31.050	-9.230
84	29848MOR	MM	31.217	-8.833
84	29850MOR	MM	31.217	-8.833
85	29851MOR	MM	31.583	-5.583
86	22936USA	SS	32.176	-110.709

Supplementary Table S1 – Cont.

87	22937USA	SS	32.205	-110.729
88	17121USA	SS	33.917	-115.917
89	1105USA	SS	34.711	-98.615
90	1096ESP	RM	36.935	-5.267
91	28952FRA	MM	45.777	0.787

Supplementary Material S2 – Ribotype groups and samples.

R1: ARG18294, ARG18119, ARG18260, ARG18095, ARG18090, ARG18092, ARG18093, ARG19483, ARG18096, ARG18133, ARG18135, ARG18269, ARG18392, ARG18393
R2: ARG18091, ARG18336
R3: ARG18097
R4: ARG18695
R5: CHI15555
R6: CHI15541, CHI15560
R7: CHI15652
R8: CHI15613, CHI15679, CHI15680, CHI15674, CHI17985, CHI17940, CHI15615, CHI17980, CHI19113
R9: CHI15638, CHI19117
R10: CHI15695, CHI17964, CHI17932, CHI17522, CHI15668, CHI17523, CHI17989
R11: ARG18129, ARG18121, ARG19481, ARG19486, ARG18120, MOR35706MM, MOR35713MM, MOR35739MM, MOR35775MM
R12: MOR29850MM, MOR35733MM
R13: CHI15619
R14: FR28952MM
R15: MADA19955, MADA19958, USA22936SS
R16: MOR21735MM, MOR29848MM, MOR29851MM, MOR35700MM, MOR35708MM, MOR-35723MM, MOR35724MM, MOR35728MM, MOR35729MM, MOR35767MM, MOR35772MM
R17: MEX10760, MEX11094, MEX14969
R18: USA17121SS
R19: MEX14750, MEX12732, MEX12741, MEX10910, MEX11339, MEX11149, MEX12744, MEX13009, MEX18822, MOR35766MM
R20: MEX12731
R21: MEX15037, MEX18940, ESP1096RM
R22: MEX12922, USA879SS
R23: USA807SS
R24: MEX14763
R25: MEX12984
R26: MEX15182
R27: MEX14741
R28: MEX14789, MEX14942, MEX10927, MEX12704, MEX12768, MEX18857, MEX35326MM, GBR22029SS, USA1105SS
R29: MEX11257, MEX14734
R30: BRA60295LHC
R31: MADA19860, USA22937SS
R32: MEX11251, MEX11099
R33: CAIS13114, MADA19834, MADA19859, MADA19879, MADA19990, MADA19995, MADA19914, MADA19989, MADA19916, MADA19940, MEX18858, BRA60296LHC
R34: MADA19986
R35: MEX14933
R36: MEX14951
R37: MEX14995, MEX15003